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E1.28: PNL-4100/pt

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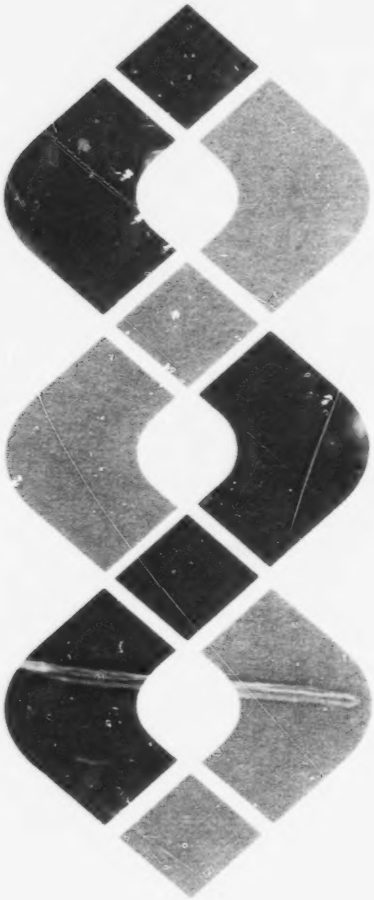
Pacific Northwest Laboratory Annual Report for 1981 to the DOE Office of Energy Research

Part 1 Biomedical Sciences February 1982



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Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
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PNL 1981 Environment, Health and Safety Part 1

N O T I C E

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PACIFIC NORTHWEST LABORATORY
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for the
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under Contract DE-AC06-76RLO 1830

Printed in the United States of America
Available from
National Technical Information Service
United States Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22151

NTIS Price Codes
Microfiche A01

Printed Copy

Pages	Price Codes
001-025	A02
026-050	A03
051-075	A04
076-100	A05
101-125	A06
126-150	A07
151-175	A08
176-200	A09
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Pacific Northwest Laboratory Annual Report for 1981 to the DOE Office of Energy Research

Part 1 Biomedical Sciences

H. Drucker and Staff Members
of Pacific Northwest Laboratory

February 1982

Prepared for
the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

II

PREFACE

This 1981 annual report from Pacific Northwest Laboratory (PNL) to the Department of Energy (DOE) describes research in environment, health, and safety conducted during fiscal year 1981. The report again consists of five parts, each in a separate volume.

The five parts of the report are oriented to particular segments of our program. Parts 1 to 4 report on research performed for the DOE Office of Health and Environmental Research in the Office of Energy Research. Part 5 reports progress on all research performed for the Office of the Assistant Secretary for Environmental Protection, Safety and Emergency Preparedness. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the interdisciplinary nature of the research effort. Project reports in Parts 1 to 4 are organized primarily by energy technology.

The parts of the 1981 Annual Report are:

Part 1: Biomedical Sciences
Program Manager - H. Drucker — D. L. Felton, Editor

Part 2: Ecological Sciences
Program Manager - B. E. Vaughan — B. E. Vaughan, Report Coordinator
C. M. Novich, Editor

Part 3: Atmospheric Sciences
Program Manager - C. E. Elderkin — R. L. Drake, Report Coordinator
N. M. Burleigh, Editor

Part 4: Physical Sciences
Program Manager - J. M. Nielsen — J. M. Nielsen, Report Coordinator
I. D. Hays, Editor

Part 5: Environmental and Occupational
Protection, Assessment,
and Engineering
Program Managers - D. L. Hessel — W. A. Glass, Report Coordinator
S. Marks — R. W. Baalman, Editor
W. A. Glass

Activities of the scientists whose work is described in this annual report are broader in scope than the articles indicate. PNL staff have responded to numerous requests from DOE during the year for planning, for service on various task groups, and for special assistance.

The topic of the 21st Hanford Life Sciences Symposium, which was held this year, was "Biological Availability of Trace Metals: Chemical Estimation, Ecological and Health Implications."

A highlight of the year was completion of the third floor of the office wing of the Life Sciences Laboratory. This opens up substantial laboratory space by allowing investigators to move offices from the laboratory to the new area.

Credit for this annual report goes to many scientists who performed the research and wrote the individual project reports, to the program managers who directed the research and coordinated the technical progress reports, to the editors who edited the individual project reports and assembled the five parts, and to Ray Baalman and Irene D. Hays, editors in chief, who directed the total effort. The size of this year's report has been reduced as the result of an effort to report more fully in the open literature.

W. J. Bair, Manager
S. Marks, Associate Manager
Environment, Health and Safety Research
Program

Previous Reports in this series:

Annual Report for

1951	W-25021, HW-25709
1952	HW-27814, HW-28636
1953	HW-30437, HW-30464
1954	HW-30306, HW-33128, HW-35905, HW-35917
1955	HW-39558, HW-41315, HW-41500
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1960	HW-69500, HW-70050
1961	HW-72500, HW-73337
1962	HW-76000, HW-77609
1963	HW-80500, HW-81746
1964	BNWL-122
1965	BNWL-280; BNWL-235, Vol. 1-4; BNWL-361
1966	BNWL-480, Vol. 1; BNWL-481, Vol. 2, Pt. 1-4
1967	BNWL-714, Vol. 1; BNWL-715, Vol. 2, Pt. 1-4

1968	BNWL-1050, Vol. 1, Pt. 1-2; BNWL-1051, Vol. 2, Pt. 1-3
1969	BNWL-1306, Vol. 1, Pt. 1-2; BNWL-1307, Vol. 2, Pt. 1-3
1970	BNWL-1550, Vol. 1, Pt. 1-2; BNWL-1551, Vol. 2, Pt. 1-2
1971	BNWL-1650, Vol. 1, Pt. 1-2; BNWL-1651, Vol. 2, Pt. 1-2
1972	BNWL-1750, Vol. 1, Pt. 1-2; BNWL-1751, Vol. 2, Pt. 1-2
1973	BNWL-1850, Pt. 1-4
1974	BNWL-1950, Pt. 1-4
1975	BNWL-2000, Pt. 1-4
1976	BNWL-2100, Pt. 1-5
1977	PNL-2500, Pt. 1-5
1978	PNL-2850, Pt. 1-5
1979	PNL-3300, Pt. 1-5
1980	PNL-3700, Pt. 1-5

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FOREWORD

This volume describes progress on biomedical and health effects research conducted at PNL in 1981. The contents of the volume are a reflection of our continuing emphasis on the evaluation of risk to man from existing and/or developing enery-related technologies. The emphasis of the PNL program is consistent with the DOE goal of increasing and diversifying national energy resources without increasing risks to human health.

Most of the studies described in this report relate to activities for four major energy technologies; nuclear fuel cycle; fossil fuel cycle (oil, gas, and coal process technologies, mining, and utilization; synfuel development), geothermal power production; and fusion (biomagnetic effects). The report is organized under these technologies. In addition, research reports are included on the application of nuclear energy to biomedical problems.

Technically, the energy-related projects presented here all center around a common research format involving multitiered toxicologic evaluation of potentially hazardous by-products, fugitive gases and effluents from energy activities. The coal liquefaction research project, for example, is illustrative of the multitiered toxicologic concept. Products and process streams are examined by an inexpensive microbial mutagenesis assay. The results of these investigations are used to set priorities for materials to be used in the more expensive animal carcinogenicity and teratogenicity test systems. The initial acute animal studies, in turn, are used to identify the need for examining noncarcinogenic effects, such as damage to respiratory, neurologic, and immunologic systems.

The qualitative validity of applying results from animal experimentation to man is firmly based on empirical observations. However, as indicated in reports on animal lifespan studies associated with nuclear fuel cycles, some progress is being made in obtaining data which will provide a more quantitative basis for the extrapolation of animal data to man.

Major progress is being made in determining potential health effects of coal liquids relative to other fossil fuels and synfuels such as shale oil. In particular, work in progress is leading to some understanding of the specific chemical entities that might be responsible for the biological effects of the complex mixtures that are coal liquids.

The biomedical and health effects program at PNL is an interdisciplinary effort requiring scientific contributions from practically all research departments at PNL. The personnel in the Biology Department are the principal contributors to the volume. Requests for reprints from the list of publications for 1981 on pages 167 to 173 will be honored while supplies last.

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Coal

XII

COAL

Studies of the area of coal energy technology are relevant to potential occupational and environmental health concerns of coal combustion and coal liquefaction.

In the area of coal liquefaction a tiered testing approach is used, going from cellular systems to mammalian acute and chronic toxicity assays and examining systemic/ mutagenic potential of materials that may be of occupational and/or environmental health concern.

Toxicologic studies deal with the potential hazard to miners of elements found in mine atmospheres, and with effects of inhaled heavy metals that may be present in fly ash.

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• Mutagenicity of SRC Materials

Principal Investigator: R. A. Pelroy

Other Investigators: P. S. Lacy, T. L. Mohr, and D. L. Stewart

Detailed experimental results, and discussion of their implications, are presented in "Fractional Distillation as a Strategy for Reducing the Genotoxic Potential of SRC-II Coal Liquids: A Status Report," PNL-3787. Therefore only the following summary is presented here.

The genotoxicity of coal liquids produced by direct liquefaction in the solvent refined coal-II (SRC-II) process is found only in the heavy-distillate fraction, i.e., components with boiling points (BP) greater than or equal to 550°F. This is not surprising, since the large-ring, high-boiling polycyclic compounds in these complex hydrocarbons probably account for most of their genetic activity. Therefore, we felt that it might be possible to partition various coal liquids into genetically active and inactive materials by fractional distillation. This possibility was investigated in the following way: Two sets of full-boiling-range SRC-II materials, each representing independent process runs, and each covering a temperature range from 300-850°F, were fractionally distilled into BP cuts of 50°F or less. Fractional distillation was carried out with laboratory equipment that may resemble a similar unit operation for the SRC-II process.

The BP cuts were subjected to analysis for genotoxicity in a battery of microbial (in vitro) screening tests: Salmonella typhimurium mutagenesis tests (Ames histidine reversion and forward mutation to 8-azaguanine resistance) and Escherichia coli primary DNA damage (prophage induction). These bioassays were carried out on both the crude BP cuts and also on their chemical class fractions, provided by high-pressure liquid chromatography. Only compounds in the full-boiling-range material with BP of 650°F or above were active in the bioassay systems. Most of the activity (>80%) was in constituents boiling above 800°F, and was contained in about 8% (by weight) of the full-boiling-range material.

The Ames-assay-demonstrated mutagenicity of the BP cuts was nearly abolished by treatment with nitrous acid, suggesting that polyaromatic amines (PAA) are the determinant mutagens in these materials over the entire BP range where genotoxicity is detected. Chemical analysis of the BP cuts supported this hypothesis, showing increased concentration of 4-ring PAAs in the most genetically active BP cuts. In relative terms, the three microbial tests were in nearly quantitative agreement in showing the most genetic activity in BP cuts above 850°F.

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• Solvent Refined Coal Biostudies

Principal Investigator: D. D. Mahlum

Other Investigators: T. K. Andrews, M. E. Frazier, B. J. Kelman, S. M. Loscutoff, R. E. Schirmer, D. L. Springer, B. A. Vieux, and R. B. Westerberg

Technical Assistance: J. A. Cushing, C. J. Gerdes, B. W. Killand, L. W. McGee, L. F. Montgomery J. E. Samuel, and D. C. Snyder

This project has examined the toxicologic properties of a number of SRC-derived materials. Several have been shown to possess various kinds of biological activities. In the studies to be reported here, we will describe results obtained from studies with mammalian cell culture, the initiation-promotion assay, from inhalation studies, and from efforts at establishing the dosimetric basis for some of the results produced by components of coal-derived materials.

Cell Transformation Studies

The Ames assay has been used to screen a large number of coal-derived materials to establish a large data base on microbial mutagenesis. It is now desirable to further examine many of these materials in a higher-order test which utilizes mammalian cells. This is particularly, true since the ultimate goal of our in vitro studies is to use them as predictors of the carcinogenicity of complex organic materials. Our second tier of evaluation is the Syrian hamster embryo (SHE) cell transformation assay. The advantage of this assay is that it uses mammalian cells but is more rapid than whole-animal assays. By means of the SHE assay, we have evaluated a number of coal-derived liquids, and their fractions, to correlate results with those of the Ames assays and in vivo animal carcinogenesis studies.

Two sets of fractionally distilled coal-liquid samples were studied. Sample Set No. 1 comprised 18 distillate cuts with boiling points between 138° and 1,055°F. Several boiling-point ranges overlapped; we examined eight representative cuts with boiling points between 640° and 1,060°F. The second set consisted of distillate cuts with boiling points ranging from 300-850°F. These cuts represented 50° increments in boiling points in the range 700-850°. We also determined the effect of hydrotreatment on the transforming activity of a coal-derived fuel blend.

The materials were incubated with second-passage SHE cells in the presence of S9, a fraction of rat liver homogenate containing enzymes active in metabolism of aromatic compounds. After passages, the cells were scored for altered morphology.

The results of the SHE-cell assay with distillate cuts from Sample Set No. 1 are shown in Table 1. A very low level of activity was found for cuts boiling between 640 and 780°F; however, activity increased dramatically for cuts boiling above 870°. Similar results were obtained for Sample Set No. 2 (Table 2). Activity was found only at boiling points exceeding 850°F.

TABLE 1. Response of Syrian Hamster Embryo Cells to Boiling-Point Fractions Prepared from SRC-II Liquid (Sample Set No. 1; See Text).

Boiling Point Range, °F	Transformants/µg Test Material
640-690	0.04
710-830	0.03
710-730	0.03
740-760	0.04
650-780	0.08
780-880	0.08
870-970	0.66
910-1060	0.94
Controls	
DMSO	<0.001
BaP	0.80
2-Aminoanthracene	0.26

These results are qualitatively similar to those previously reported by Pelroy using

TABLE 2. Response of Syrian Hamster Embryo Cells to Fractionally Distilled Coal Liquid (Sample Set No. 2; See Text).

Boiling Point Range, °F	Transformants/µg Test Material
387-700	0.05
700-750	0.08
750-800	0.11
800-850	0.13
850⁺	0.75
Controls	
DMSO	0.03
Crude Petroleum	0.06
BaP	0.65
2-Aminoanthracene	0.17

the Ames test (PNL-3787), although low-level activity was observed in the lower-boiling-point cuts with the SHE-cell assay which was not found with the Ames assay. It is not clear at this time whether this low level of activity is significant relative to the carcinogenic potential of these cuts.

Table 3 shows that the transforming activity of an SRC-II fuel-oil blend was reduced 50-75% by hydrotreatment. This reduction, although substantial, is not as great as that found for microbial mutagenic activity.

TABLE 3. Response of Syrian Hamster Embryo Cells to Hydrotreated SRC Materials.

Material	Transformants/µg Test Material
Feedstock	0.45
Medium Hydrotreatment	0.11
Severe Hydrotreatment	0.19
Controls	
DMSO	<0.001
BaP	0.72
2-Aminoanthracene	0.17

Initiation-Promotion Studies

Efforts to evaluate the in vitro assays for their ability to predict carcinogenicity have been hampered by the protracted length of time and the large amounts of material required for long-term skin assays. We have therefore begun to evaluate the initiation-promotion (I/P) assay for its usefulness in studying the carcinogenicity of coal-derived liquids.

In our first experiment, we tested heavy distillate (HD) and several of its fractions, since these materials had been assayed in Ames, SHE and long-term skin-painting assays. Fifty-µl doses of HD (or of fractions of HD) were applied to the shaved backs of male CD-1 mice (Charles River). Two weeks later, twice-weekly applications of phorbol myristate acetate (PMA; 5 µg/application) were begun. Negative controls received acetone as the initiator. Two groups of positive controls were used: One group was initiated with benzo[a]pyrene (BaP), the other with dimethylbenzanthracene (DMBA). In addition, two groups were treated with materials derived from the fractional distillation of a wide-boiling-point-range coal liquid: One group received 50 µl of material that boiled from 300-700°F; the other received material that boiled between 800-850°F. All groups received the same regimen of PMA treatment as the mice initiated with HD or its fractions.

The DMBA-treated group had the greatest positive response: All animals developed papillomas (Figure 1) at a very rapid rate. The responses for BaP, HD, neutral tar, and basic tar groups were similar to one another. Responses were somewhat lower with the basic and polynuclear aromatic (PNA) fractions. No response was observed in animals that had been initiated with 300-700°F material; a moderate response was seen in those initiated with the 850°F-boiling-point cut.

In the next experiment, additional materials derived by fractional distillation were tested. Groups of 30 female CD-1 mice received initiating doses of cuts boiling from 700-750°, 750-800°, 800-850°, or of a cut with the wide-boiling-point-range of 300-850°F. The results are shown in Figure 2. There was a very low response to the 700-750° and the 750-800° material, a moderate response for the 800-850° cut and an intermediate response with the 300-850° material.

Although this experiment is still in progress, results obtained to date suggest that the I/P assay may aid in identifying

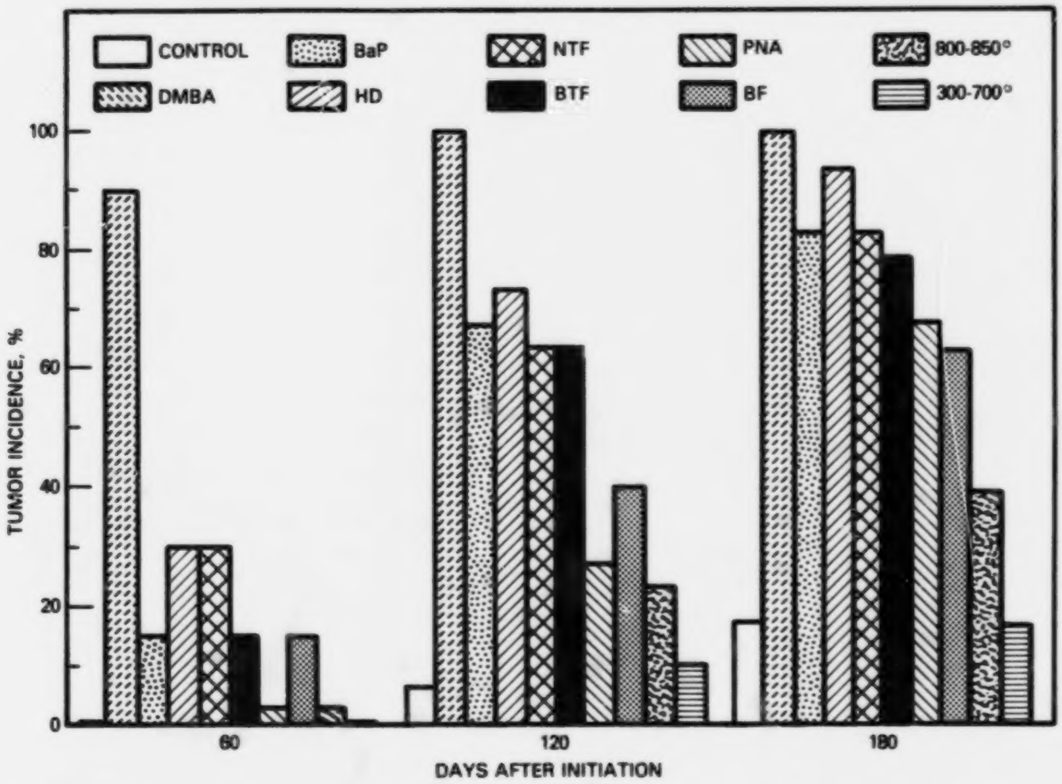

FIGURE 1. Effectiveness of Various Coal-Derived Liquids as Initiators of Skin Tumorigenesis. Abbreviations: DMBA, dimethylbenzanthracene; BaP, benzo[a]pyrene; HD, heavy distillate; NTF, neutral tar fraction of HD; BTF, basic tar fraction of HD; PNA, polynuclear aromatic fraction of HD; and BF, basic fraction of HD. The 300-700°F and 800-850°F materials are distillation cuts obtained from a coal liquid fuel oil blend.

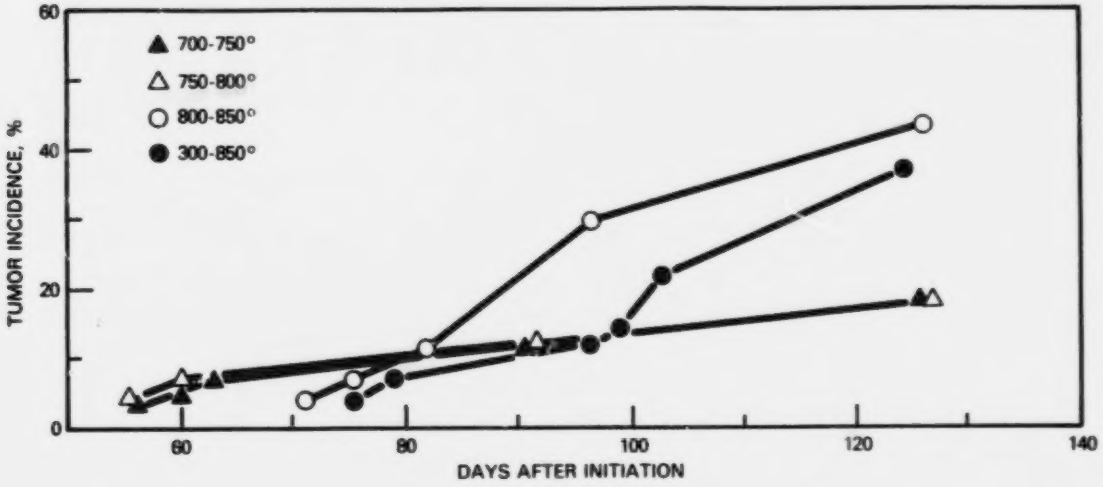

FIGURE 2. Effectiveness of Various Coal-Liquid Distillation Cuts (°F) as Initiators of Skin Tumorigenesis.

the constituents of complex organic materials responsible for carcinogenicity. Each material which has shown a positive response in the long-term skin-painting assays also showed a positive response in the I/P assay.

Inhalation Studies

Further improvements have been made in the generation and delivery systems for coal-liquid aerosols. The Retec generator used previously has occasionally plugged, necessitating filtration of the coal liquid prior to generation, as well as frequent and thorough cleaning of the generator. The Solo-Sphere® generator has, therefore, been evaluated as an alternative to the Retec. Tests indicate that suitable and stable concentrations of coal-liquid aerosols can be achieved for prolonged periods without prior filtration of the generating liquid. The mass mean aerodynamic diameter of the aerosol produced by the Solo-Sphere® was approximately 1.7 µm, with a geometric standard deviation of 2.2.

An aerosol delivery system has been designed and fabricated which provides aerosol to several exposure chambers in various concentrations (Figure 3), employing a manifold attached directly to the generating system. The manifold delivers aerosol to each exposure chamber through a tube which has an orifice with a diameter specially designed to provide the desired concentration. The orifice diameters were calculated using the practical working equation adopted by the Research Committee on Fluid Meters of the American Society of Mechanical Engineers. Precise control of aerosol concentration required additional fine adjustment of the orifice diameter.

Aerosol samples of SRC-I process solvent (PS), generated and delivered as described above, were fractionated with a Mercer cascade impactor and the UV absorption spectra of material collected on each stage were determined. The spectra of the material collected in a chloroform trap were similarly determined. Both the material from the last stage of the impactor (a filter) and that from the solvent trap had

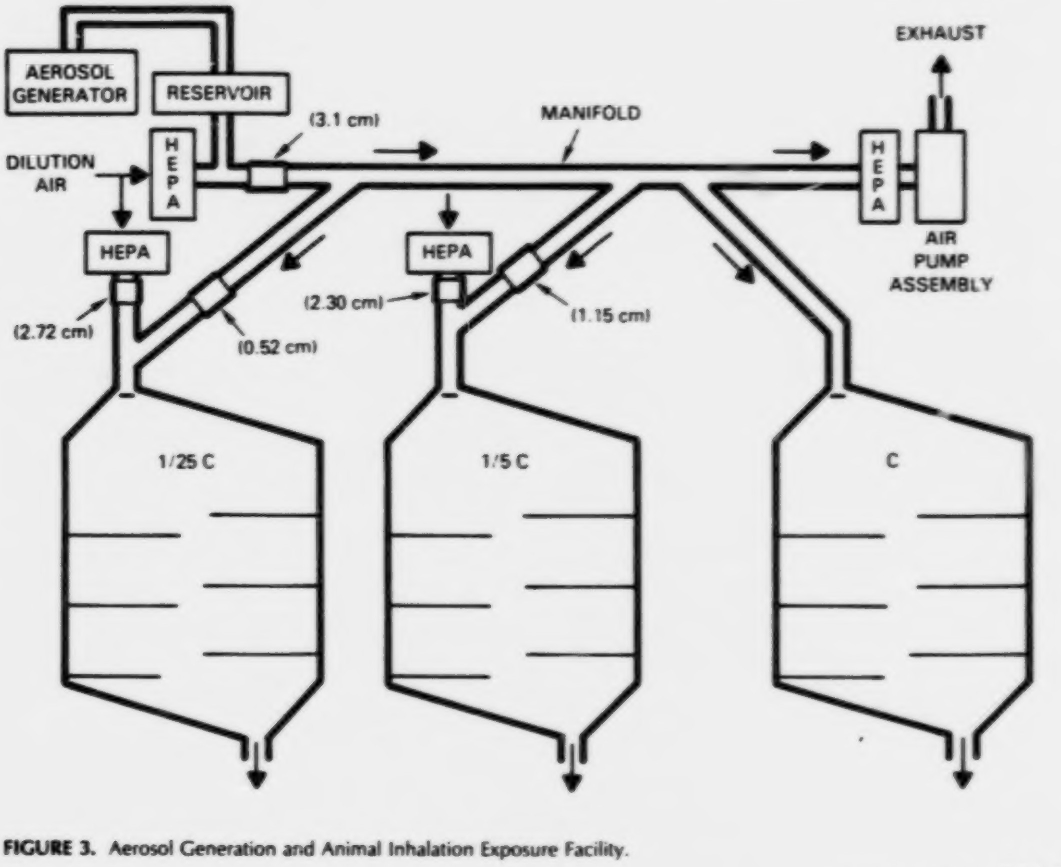

FIGURE 3. Aerosol Generation and Animal Inhalation Exposure Facility.

a major absorption peak at 276 nm, indicating the presence of 2-ring PNA compounds such as naphthalenes and substituted naphthalenes. In contrast, the absorption peak for material from the other stages of the impactor, and for the original liquid, was at 254 nm, suggestive of 3-ring (or larger) PNAs.

We had previously found (Proceedings, 20th Hanford Life Sciences Symposium; in press) that exposing guinea pigs to approximately 0.2 mg/L of SRC-I PS increased their sensitivity to histamine challenge. We therefore performed an experiment to determine if exposure to SRC-II HD aerosol produced a similar effect. Eight-week-old guinea pigs were exposed to 0.16 mg/L of HD for 8 days. Twenty-four hours later, they were challenged with histamine, and pulmonary resistance and compliance were measured. Resistance increased and compliance decreased (Figures 4 and 5) in HD-exposed animals compared to the controls. Histamine challenge thus appears to be a promising method for examining pulmonary damage in guinea pigs. In rats, however, as we have shown in other studies in our laboratory, histamine challenge is not a sensitive test of pulmonary functions.

Placental Transfer Studies

Although high-boiling-point coal liquids have been shown to be teratogenic (PNL-3189), no information was available concerning the ability of various constituents to cross the placenta. We have therefore used a placental perfusion system to measure fetal exposure to BaP, a constituent of coal liquids, in an initial attempt to understand the pharmacokinetic of cross-placental transfer of organic materials.

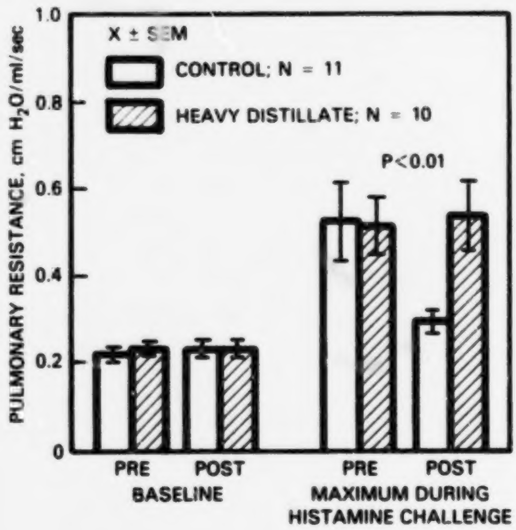

FIGURE 4. Pulmonary Resistance (cm H_2O/ml/sec) Following 12-Day Exposure to 0.084 mg/L Heavy Distillate.

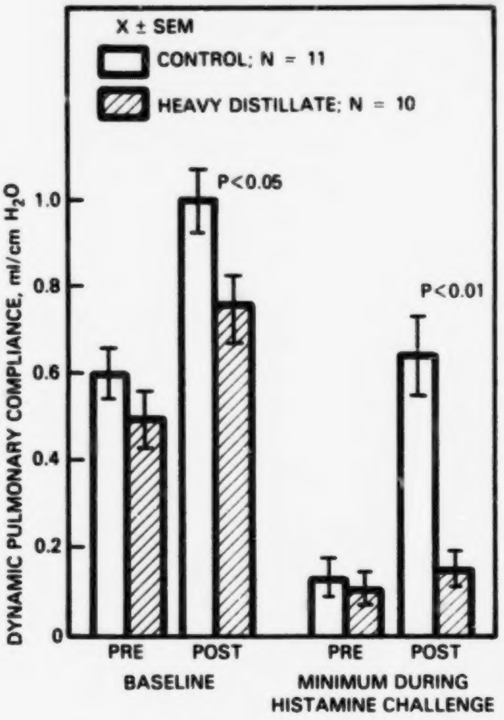

FIGURE 5. Dynamic Pulmonary Compliance (ml/cm H_2O) Following 12-Day Exposure to 0.084 mg/L Heavy Distillate.

The clearance of radiolabeled BaP from dam to fetus was measured across the hemochorial placenta of the guinea pig at 60 days of gestation. Using techniques previously reported for other toxic materials, the fetal circulation of the placenta was isolated, BaP was injected into the maternal circulation, and the concentration of BaP was determined in the perfusate. Clearance of BaP from dam to fetus, which was high (Table 4) following intravenous injection, appeared to be a function of umbilical blood flow, ranging from 0.59 to 2.40 ml/min at an umbilical flow rate of 2.5 ml/min. The clearance rates of BaP approximated those for tritiated water, indicating that BaP easily crosses the placenta.

TABLE 4. Benzo[a]pyrene Clearance from Mother to Fetus.

		Clearance Measurements, ml/min					
		^{3}H-Labeled Water		Benzo[a]pyrene			
						Correlation with T_{MF}	
Dam No.	Placenta No.	At 2.5 ml/min(a), ± SE	Range	At 2.5 ml/min(a), ± SE	Range	r(b)	P
1	1	--	--	0.60 ± <0.01	0.26-0.71	--	--
2	1	3.25	0.33-0.71	2.40	0.71-1.10	0.99	<0.001
3	1	1.72 ± 0.10	0.39-1.74	1.62 ± 0.11	0.31-1.64	0.98	<0.001
4	1	1.14 ± 0.09	0.54-1.92	1.09 ± 0.07	0.72-1.53	0.99	<0.001
4	2	1.16 ± 0.05	0.65-1.15	1.03 ± 0.06	0.44-1.00	0.97	<0.01
5	1	2.08 ± 0.10	1.52-2.61	2.62 ± 0.41	2.08-3.32	0.67	<0.05
6	1	2.51 ± 0.06	1.69-2.15	2.67 ± 0.25	0.70-4.31	0.95	0.001
7	1	0.58 ± 0.02	0.28-0.43	0.30 ± 0.02	0.16-0.26	0.98	0.001

(a) As predicted from the regression of T_{MF} or BaP_{MF} on umbilical flow
(b) Correlation coefficient for the regression of BaP_{MF} on T_{MF}

• Health Effects of Synthetic Fuels

Principal Investigator: R. A. Renne

Other Investigators: L. G. Smith and H. D. Tolley

Technical Assistance: S. M. Baze, J. C. Chapman, V. L. Dedmond, S. R. Eldridge, B.D. Holloway, and B. B. Paas

The purpose of this project is to study the potential human health hazards associated with synthetic fossil-fuel technologies. Studies in progress are investigating the carcinogenic potential of cutaneous exposure to various chemically derived fractions or boiling-point distillates of materials and products from the solvent-refined coal technology.

Epidermal carcinogenesis studies in mice involving materials from several fossil-fuel technologies have recently been completed. Materials, methods, and preliminary results were reported (Annual Report, 1979, 1980). Final incidence and latency data on microscopically confirmed epidermal tumors are presented in Table 1.

Figure 1 graphically presents epidermal tumor latency data for each group, using the nonparametric Kaplan-Meier procedure. These data confirm conclusions presented earlier (Annual Report, 1980) regarding carcinogenic potency of the crude materials studied: solvent refined coal (SRC-II) heavy distillates (HD) demonstrated

TABLE 1. Incidence and Latency Data on Microscopically Confirmed Epidermal Tumors Observed in Mice Exposed to Crude Fossil-Fuel Materials or Known Carcinogens.

Material	Concentration, mg/50 µl	Mice per Group at Start of Study	Mice with Epidermal Tumors	Final Epidermal Tumor Incidence, %[a]	Minimum Time to Observed Tumor, days	Median Time to Observed Tumor, days	Maximum Time to Observed Tumor, days
Untreated	Control	50	0	0	--	--	--
Acetone	Vehicle	50	0	0	--	--	--
Shale Oil	0.21	50	0	0	--	--	--
	2.1	50	41	82	148	358	550
	21.1	50	36	72	95	200	409
Crude Petroleum	0.17	50	0	0	--	--	--
	1.7	50	10	20	282	501	708
	16.8	50	39	78	260	442	680
SRC-II Heavy Distillate	0.23	50	22	44	282	542	736
	2.3	50	45	90	72	296	372
	22.8	50	46	92	56	95	147
SRC-II Light Distillate	0.2	50	0	0	--	--	--
	2.0	50	0	0	--	--	--
	20.0	50	0	0	--	--	--
2-Aminoanthracene	0.005	50	30	60	465	583	736
	0.05	50	22	44	115	386	485
Benzo[a]pyrene	0.0005	50	0	0	--	--	--
	0.005	50	46	92	232	358	519
	0.05	50	50	100	106	143	193

(a) This does not take into account interim mortality, i.e., mice dying without epidermal tumors during the study.

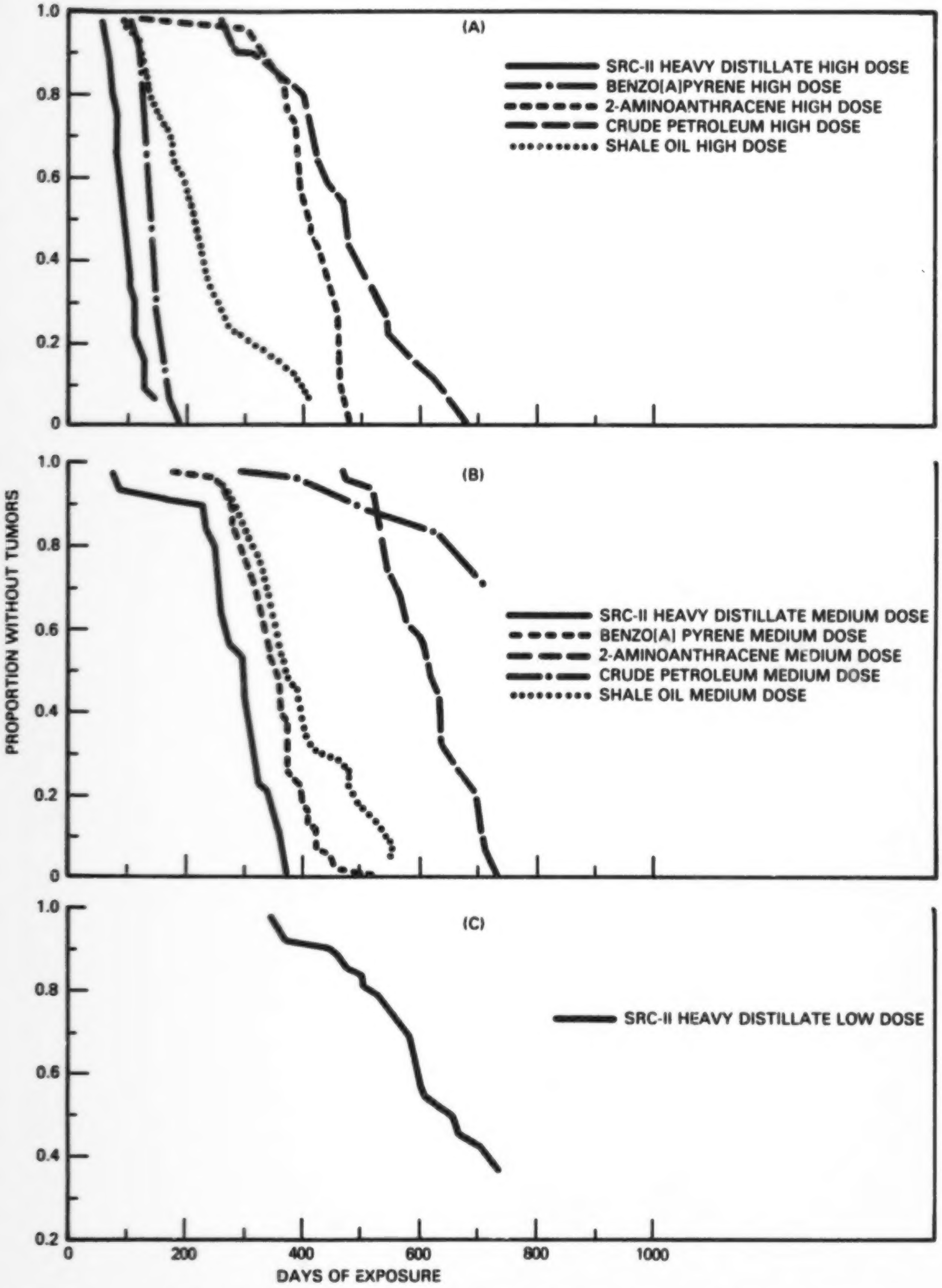

FIGURE 1. Proportion of Mice Surviving Without an Epidermal Tumor During Three-Times-Weekly Exposure to Crude Fossil Fuel Materials [(A) High Dose, (B) Medium Dose and (C) Low Dose] or Known Carcinogens; Kaplan-Meier Estimates.

the most carcinogenic activity, followed (in decreasing order) by crude shale oil and crude petroleum.

Similar epidermal carcinogenesis studies are in progress on chemically derived fractions and boiling-point distillates of materials and products from the SRC technology. Details of the sources of the chemically derived fractions of SRC-II HD, the experimental methods used, and initial results of studies on chemical fractions were presented previously (Annual Report, 1980). Samples of five boiling-point distillates (cut 1: 300-700°F; cut 2: 700-750°F; cut 3: 750-800°F; cut 4: 800-850°F; bottoms: >850°F), and composite product (300 to >850°F) from the SRC plant at Harmarville, PA were obtained from Gulf Research and Development Co., Pittsburgh, PA. All synthetic fuel materials used as the source of these fractions and boiling-point distillates were derived from experimental units and may not be representative of commercial practice.

Updated tumor incidence and latency data on skin tumors observed at gross examination in mice exposed to chemical fractions of SRC-II HD are presented in Table 2 and Figures 2 and 3. These updated data confirm earlier findings (Annual Report, 1980) that the basic tar and neutral tar are the most carcinogenically active fractions tested, from the standpoint of skin tumor latency and incidence. The PNA fraction shows much less carcinogenic activity than the tar fractions. Basic fraction has relatively low carcinogenic activity in this assay system; skin tumors were observed only in the group exposed to the highest dose (see Annual Report, 1980).

Skin-painting assays of boiling-point distillates from the product of the Harmarville, PA SRC plant began only recently; no skin-tumor incidence data are available yet from these studies.

Future work on this project will examine, in our mouse skin-painting assay system, the carcinogenicity of recombined chemical fractions of SRC-II HD, and the effects of destruction of aromatic amine components of fractions by nitrosation, on the carcinogenicity of these fractions.

TABLE 2. Incidence of Grossly Observed Skin Tumors in Mice After Exposure to Chemical Fractions of SRC-II Heavy Distillate.

Material	Dose Group	Days on Study	Number Mice at Risk[a]	Mice with Skin Tumors	Tumor Response, %	Minimum Latency, days	Median Latency, days	Maximum Latency, days
30% MeOH[b]	Vehicle	736	8	0	0	--	--	--
Replication	Medium	437	49	49	100	163	291	354
SRC-II Heavy Distillate	High	323	50	50	100	44	112	232
SRC-II Heavy Distillate								
Basic Fraction	Low	735	11	0	0	--	--	--
Basic Fraction	Medium	735	16	0	0	--	--	--
Basic Fraction	High	735	34	34	100	323	582	722
SRC-II Heavy Distillate								
PNA Fraction	Low	735	22	5	23	393	--	--
PNA Fraction	Medium	606	46	46	100	239	428	568
PNA Fraction	High	451	49	49	100	171	267	351
SRC-II Heavy Distillate								
Neutral Tar	Low	656	37	34	92	240	561	--
Neutral Tar	Medium	452	49	49	100	177	281	372
Neutral Tar	High	332	50	50	100	99	183	235
SRC-II Heavy Distillate								
Basic Tar	Low	619	44	40	91	239	435	--
Basic Tar	Medium	389	49	49	100	162	252	309
Basic Tar	High	291	50	50	100	74	148	204

(a) Number of mice at risk excludes those that died without developing skin tumors, and includes all live animals in the group plus dead animals that had skin tumors.

(b) 30% MeOH methanol in acetone, 30% (V/V)

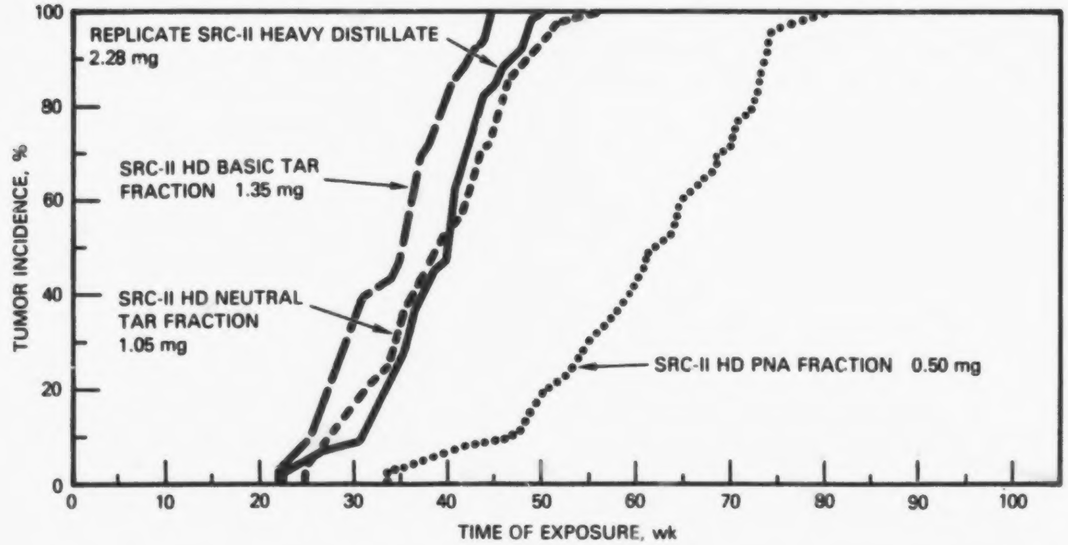

FIGURE 2. Skin Tumor Incidence Versus Time of Exposure of Mice to Middle Doses of SRC-II Heavy Distillate Fractions.

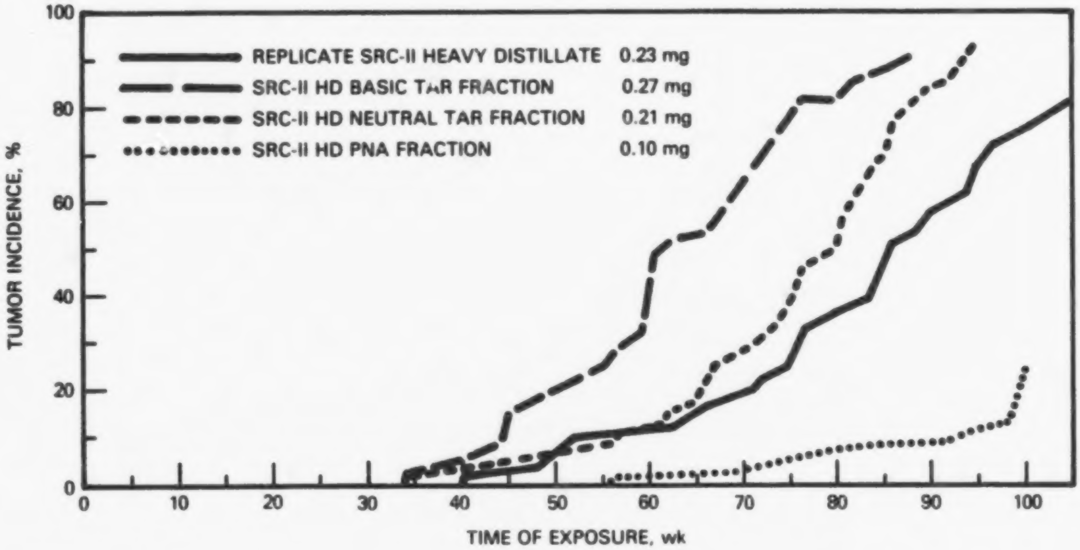

FIGURE 3. Skin Tumor Incidence Versus Time of Exposure of Mice to Low Doses of SRC-II Heavy Distillate Fractions.

• Perinatal Effects of SRC

Principal Investigator: D. L. Springer

Other Investigators: F. D. Andrew and B. J. Kelman

Technical Assistance: P. S. Lytz

Studies were conducted to determine whether in utero exposure of rats to high-boiling coal liquid caused adverse effects relative to the following endpoints: survival, body weight, immune system development, metabolic enzyme activity, and behavior. The data indicate that the treatment increased the incidence of fetal and neonatal mortality. Body weights at 1 day of age were significantly less in the heavy-distillate-(HD)-treated groups given 0.3 and 0.6 g/kg body weight/day and in the process-solvent-(PS)-treated group at 0.6 g/kg/day. Perinatal growth was retarded through 21 days of age in the HD- and PS-treated groups at the highest doses and through 7 days of age in the HD group given 0.3 g/kg/day. Immunocompetence also was altered by exposure to these materials. Few changes were observed either in enzyme activities or behavior; changes in these parameters were less sensitive as measures of effects than survival and weight gain.

Prenatal exposure to high-boiling materials from coal liquefaction processes is teratogenic in the rat, causing cleft palate, small lungs, and diaphragmatic hernia. Fetuses with these malformations would probably not survive outside the uterus. In addition, other, less-severe, morphological or functional impairments resulting from exposure may lead to impaired postnatal function. Therefore, we have conducted studies to determine whether pregnant rats treated with coal-liquid materials produce offspring that are adversely affected. The biological endpoints for these studies include: 1) survival for the first 24 hr after birth; 2) body weight gain through 21 days of age; 3) immunocompetence for both humoral and cell-mediated systems; 4) behavioral and maturational measurements; and 5) enzyme activity as a measure of biochemical maturation.

Survival and Growth

For these studies, pregnant rats were exposed by gavage on days 12-16 of gestation. Materials tested included heavy distillate (HD) from SRC-II and process solvent (PS) from SRC-I. Aroclor 1254, a polychlorinated biphenyl, was administered in corn oil as a positive control. Two negative control groups were routinely evaluated. One group (shelf control) was not gavaged with the vehicle; this group was used for statistical comparison of the immunocompetence data. The second group was given corn oil by gavage (corn oil vehicle [COV] control). Offspring were evaluated at birth and on days 1, 7, 14, and 21 of age.

The number of live fetuses (or neonates) were determined (Table 1) at 19-21 days of gestation, at birth, and 1 day of age. On days 19-21 of gestation, litters from dams exposed to 0.15 g/kg body weight/day of HD were similar in size to those of the COV controls. In contrast, at doses of 0.3 and 0.6 g/kg/day, the number of live fetuses decreased relative to COV controls, suggesting intrauterine death in the HD-treated group. The number of live births also declined ($P \leq 0.07$) for the highest-dose HD treatment group (0.6 g/kg/day), with a commensurate increase in the number of stillbirths. Furthermore, litter size in the highest HD treatment group declined significantly between birth and 1 day of age, suggesting that many of the neonates were unable to survive outside the uterus. Similar comparisons for the PS-treated groups indicated that this material did not significantly affect intrauterine death rate or mortality rate during the first 24 hr after birth.

We also compared the survival of offspring from dams treated with coal liquid to that of those treated with Aroclor, which is known to be toxic to the fetus and neonate. Aroclor treatment did not reduce the number of live fetuses prior to birth, but about one-half were stillborn at all treatment levels. Even though a number of fetuses died during the first day after birth, the difference was not statistically significant on a per-litter basis.

Body weights for fetuses exposed in utero to 0.15 g/kg/day of HD were not significantly different from those of COV controls (Table 2). At a dose of 0.3 g/kg/day, weights were reduced at 1 and 7 days of age; however, by 14 days of age they were not significantly different from

TABLE 1. Survival of Rat Fetuses and Neonates Following In Utero Exposure to Coal Liquids$^{(a)}$.

	Shelf Control	Corn Oil Vehicle Control	Dose, g/kg body weight/day							
			Aroclor			Heavy Distillate			Process Solvent	
			0.067	0.083	0.10	0.15	0.3	0.6	0.3	0.6
No. in Litter at 19-21 Days of Gestation	--	14.5 ± 2.6	--	--	14.3 ± 3.0	13.0	10.3 ± 3.8$^{(c)}$	10.4 ± 2.3$^{(b)}$	11.6 ± 3.9	10.3 ± 5.7
No. in Litter at Birth	14.6 ± 4.4	13.3 ± 4.0	8.0 ± 6.2	7.8 ± 5.0$^{(b)}$	6.9 ± 3.2$^{(b)}$	12.6 ± 2.1	9.1 ± 4.1$^{(b)}$	6.9 ± 4.7$^{(b)}$	12.0	10 ± 4.3
No. in Litter at 1 Day of Age	14.4 ± 4.3	13.1 ± 3.8	7.7 ± 5.9	5.5 ± 4.8$^{(b)}$	6.0 ± 2.7$^{(b)}$	12.6 ± 2.1	7.9 ± 4.4$^{(b)}$	2.0 ± 1.5$^{(b)}$	12.0	9.5 ± 3.6$^{(c)}$

(a) Mean of litter means ± SD for the number of live animals per litter; statistically analyzed using a one-tailed *t*-test.
(b) $P \leq 0.01$
(c) $P \leq 0.05$

TABLE 2. Body Weight of Offspring from Pregnant Rats Exposed to Coal Liquids.

	Shelf Control	Corn Oil Vehicle Control	Dose, g/kg body weight/day						
			Aroclor		Heavy Distillate			Process Solvent	
			0.067	0.083	0.15	0.3	0.6	0.3	0.6
No. of Litters Examined	6	14	3	9	11	14	6	2	10
Body Weight, g$^{(a)}$:									
1 day	6.7 ± 0.6	6.7 ± 0.5	5.0 ± 0.7$^{(b)}$	5.0 ± 0.6$^{(b)}$	6.5 ± 0.4	6.0 ± 0.6$^{(b)}$	4.9 ± 0.4$^{(b)}$	6.3	5.6 ± 0.7$^{(b)}$
7 days	19 ± 3	19 ± 2	10 ± 2$^{(b)}$	10 ± 3$^{(b)}$	18 ± 2	16 ± 3$^{(c)}$	10 ± 2$^{(b)}$	16	14 ± 2$^{(b)}$
14 days	37 ± 4	36 ± 3	26$^{(b)}$	23 ± 8$^{(b)}$	35 ± 3	34 ± 5	21 ± 5$^{(b)}$	35	30 ± 5$^{(b)}$
21 days	59 ± 7	56 ± 4	42$^{(b)}$	36 ± 14$^{(b)}$	56 ± 2	53 ± 7	35 ± 7$^{(b)}$	53	47 ± 8$^{(b)}$

(a) Mean of litter means ± SD for body weight in grams; statistically analyzed using a one-tailed *t*-test.
(b) $P \leq 0.01$
(c) $P \leq 0.05$

those of the COV control group, suggesting that the animals had recovered from the effects of the exposure. When the HD dose was 0.6 g/kg/day, the average litter weight was consistently less than that of the COV control group throughout the perinatal period. Exposure to PS at 0.3 g/kg/day did not significantly affect average litter weight; however, at 0.6 g/kg/day, body weights were consistently below those of the COV control group. Treatment with Aroclor also caused a significant reduction in body weight that persisted through 21 days of age. Thus, body weight gain through 21 days of age relative to the COV control was inversely related to dose for both HD and PS. In addition, HD appeared to have a greater repressive effect on rat weight gain than PS.

Immunocompetence

Responses of the developing immune system to Aroclor, HD and PS were studied. Statistical comparisons were made with shelf controls since treatment with corn oil resulted in effects on the immune system. Lymphocyte function was measured by mitogen-induced lymphocyte activation assays (in vitro correlate of cell-mediated immunity), using cells from peripheral blood, spleen, and thymus in rats after in utero exposure. Mitogens used in the lymphocyte activation assays were concanavalin-A (Con-A) and phytohemagglutinin (PHA), which stimulate T-cell populations; pokeweed mitogen (PWM), which primarily stimulates B-cells in addition to a small fraction of the T-cell population; and bacterial lipopolysaccharide (LPS), which is a B-cell activating agent. In this assay, cell activation was measured by assessing the incorporation of ^{125}I·iododeoxyuridine (^{125}I·IUdR) into newly synthesized DNA during the final 20 hr of the 3-day culture period. The distribution of T-lymphocytes in spleen-cell preparations was determined by measuring the uptake of ^{3}H-uridine.

The experiment was conducted in four replicate studies in order to treat five litters with each chemical; 3-4 male offspring from each litter were used. Dams were exposed on days 12-16 of gestation to HD, PS, or Aroclor at doses of 0.3, 0.6, or 0.08 g/kg/day, respectively. At 1 day of age, litters were culled to eight pups (usually, four males and four females). At 40 days of age, the males were weighed and sacrificed, and organ weights (spleen, thymus, liver, and brain) were recorded.

Body weights for these animals at the time of sacrifice were significantly less than those of controls for all three treatment groups (Table 3). Heavy distillate caused a significant decrease in thymus weight relative to those of controls; Aroclor exposure resulted in significant reduction in thymus and an increase in brain and spleen weights. Even though body weights of animals exposed to PS were less than those of controls, PS did not cause a significant change in the weights of the organs examined.

Significant decreases in the responses of thymus cells to PHA and LPS mitogens were observed in rats exposed in utero to HD (0.3 g/kg/day). The responses of these cells to Con-A and PWM appeared less than controls, although these differences were not statistically significant (Figure 1). Spleen-cell responses of HD-exposed rats to Con-A, PHA, and PWM were decreased relative to those of shelf controls; however, these differences were not statistically significant. Treatment with HD did not result in any differences in the mitogenic responses of peripheral-blood lymphocytes. Mitogenic responses of

TABLE 3. Body and Organ Weights of 40-Day-Old Rats Exposed In Utero to Coal Liquids.

			Dose, g/kg body weight/day		
	Shelf Control	Corn Oil Vehicle Control	Aroclor 0.08	Heavy Distillate 0.3	Process Solvent 0.6
Number of Offspring	15	19	9	16	20
Number of Litters	4	5	4	4	5
Body Weight, g[(a)]	179 ± 4	171 ± 5	120 ± 10[(c)]	161 ± 5[(c)]	162 ± 5[(c)]
Tissue Weight[(b)]					
Spleen	0.28 ± 0.01	0.30 ± 0.01	0.36 ± 0.01[(c)]	0.34 ± 0.02	0.34 ± 0.01
Thymus	0.37 ± 0.02	0.40 ± 0.01	0.28 ± 0.03[(c)]	0.32 ± 0.01[(c)]	0.38 ± 0.01
Liver	4.7 ± 0.2	4.5 ± 0.2	5.0 ± 0.3	4.5 ± 0.2	4.8 ± 0.1
Brain	1.0 ± 0.02	1.0 ± 0.03	1.5 ± 0.12[(c)]	1.1 ± 0.02	1.1 ± 0.02

(a) Mean ± standard error; statistically analyzed using a two-tailed *t*-test.

(b) As percent of body weight; mean ± standard error

(c) $P \leq 0.05$

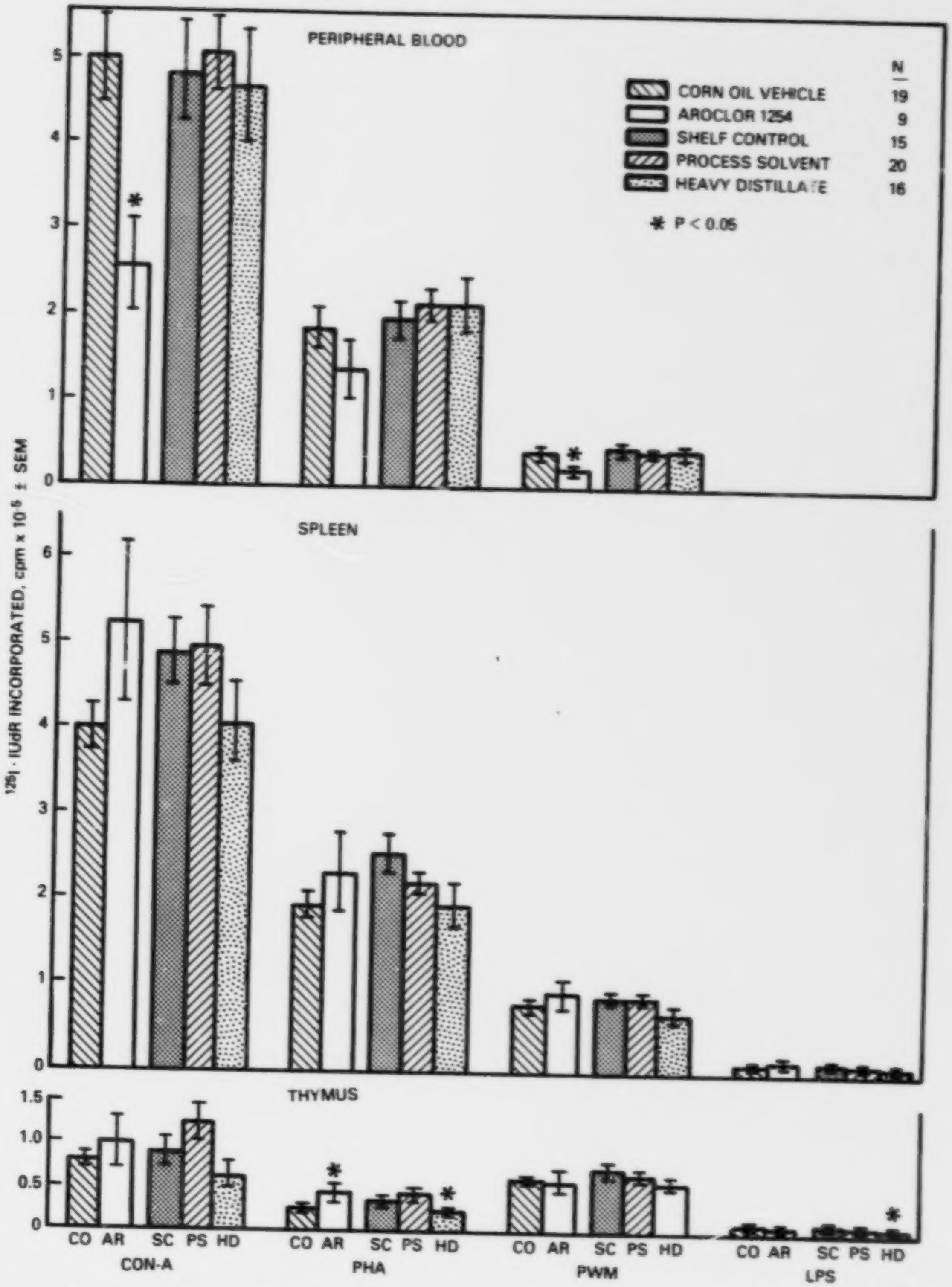

FIGURE 1. Mitogenic Responses of Peripheral-Blood Lymphocytes, Spleen and Thymus Cells of 40-Day-Old Male Rats Exposed In Utero to Coal Liquids.

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thymus, spleen or peripheral blood cells from PS-exposed (0.6 g/kg/day) rats were not altered when compared to controls. In Aroclor-exposed rats (0.08 g/kg/day), a significant decrease in peripheral-blood lymphocyte responses to Con-A and PWM were observed, as well as a significant increase in thymus cell responses to PHA.

These results indicate that HD and Aroclor decreased the mitogenic responses of both T- and B-cells in selected tissues. The consequences of these effects might be reduced cell-mediated immunity, with a potential for increased susceptibility to infection and/or neoplasia. Furthermore, exposure to HD and Aroclor significantly decreased thymus weight in these animals, possibly reducing the capability of this organ to respond to stress.

Enzyme Activities and Behavior

In an effort to obtain more sensitive measurements of toxic effects in offspring that were exposed in utero, we studied enzyme activity in several tissues. These measurements were taken at 1, 7, 14 and 21 days of age. The activities of isocitrate dehydrogenase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were determined in homogenates of liver, kidney, brain and gut. No consistent or dose-related changes were observed in enzyme activity.

Behavioral development was evaluated by forward locomotion, surface righting, air righting reflex, swimming activity, visual/vibrissal placing, forelimb strength and startle reflex. No consistent or dose-related changes were observed.

These data indicate that body weight gain, survival and immunocompetence were the most sensitive indicators of toxic effects. However, additional approaches are being considered. Further experiments are needed to substantiate the results of our studies. New biochemical studies may provide additional information on target pathway(s) responsible for the observed effects.

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• Teratology of SRC-II Materials

Principal Investigator: M. R. Sikov

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Technical Assistance: A. J. Clary, C. J. Gerdes, P. S. Lytz, R. L. Music, and S. A. Watson

Concern over the availability of adequate sources of petroleum has led to a search for alternatives, including development of coal liquefaction methods such as those in the solvent refined coal (SRC) process. Data from previous survey experiments indicated that some SRC-II materials of pilot-plant origin were embryocidal and teratogenic when administered to pregnant rats at doses approximating the maternally toxic level. This project was initiated in FY 1981 to quantify the developmental toxicity of these materials. The approach is to develop precise dose-response relationships, in rodents, to determine if levels of materials that are subthreshold for maternal toxicity are capable of affecting intrauterine growth and survival or of altering morphogenesis so as to result in malformations or other morphologic changes. These experiments may provide indications of the minimal toxic levels of SRC materials, since the embryo has been found to be a sensitive indicator of the toxicity of many substances.

Previous studies (in the project "SRC Biostudies") surveyed the embryotoxicity of several SRC-II materials after administration by gavage. Heavy distillate (HD) appeared to be the most active material; high doses produced fetotoxicity and malformations when administered daily from 12 through 16 days of gestation (dg). However, the minimal doses at which these effects (or maternal toxicity) were produced were not defined. These results, and the need for more definitive information, led to the design and execution of the project's initial two experiments, which will be described in this report. The protocol for the first experiment, using exposure by inhalation, was based on the fact that there is a potential for occupational exposure by this route, and that absorption and the associated toxicity when a material is administered by inhalation may be different from that of the same compound administered by gavage, or parenterally. The second study, using oral doses below the range which was previously studied, was based on the need to know the minimal doses at which HD produced developmental and maternal toxicities when administered by this alternative route.

Inhalation Exposure

The inhalation exposures were performed as part of a subacute toxicity study in the SRC Biostudies project; exposure details are described elsewhere in this report. Female rats (Charles River, CD) of known gestational age were randomized by weight and assigned to an exposure group and a position within the exposure chamber. The exposures were conducted as three complete replicates over a 4-wk period, to give a total of 23 to 25 pregnant animals per exposure group. Females were exposed to the HD aerosol for 6 hr/day from 12 to 16 dg. Mean aerosol concentrations during 3 wk of exposure were 0.66, 0.084, 0.017, and 0 mg HD/L of air. Rats exposed to these levels will be referred to as the high, middle, low, and control groups, respectively. Particle sizes of the aerosol for the high-, middle-, and low-concentration exposure chambers were 1.8, 1.7, and 1.6 μm mass median aerodynamic diameter (MMAD), respectively, with a geometric standard deviation (GSD) of approximately 2.0. Doses used in these experiments were substantially higher than those likely to occur for humans under normal working conditions at a coal liquefaction facility.

Animals were weighed at intervals, and sacrificed by CO_2 asphyxiation at 21 dg. Major maternal organs were weighed and observed for gross pathology. The gravid uterus, with products of conception, was also weighed. This weight was subtracted from the body weight to obtain "extragestational body weight." Changes in this measurement are considered to be a more accurate indicator of maternal effect than total body weight, since the latter is influenced by embryotoxic events. Intrauterine position and viability of the conceptuses were recorded. Fetuses and placentas were removed and weighed, and fetal crown-rump lengths were measured. Live fetuses were examined for gross defects, then evaluated for visceral malformations and altered morphologic development of the skeleton.

TABLE 1. Effects of Inhalation Exposure of Pregnant Rats to SRC-II Heavy Distillate from 12-16 Days of Gestation.

Measurement	Control	Exposure Group		
		Low	Middle	High
Number of Litters Examined	23	25	25	23
Fetoplacental Measures				
Number of Implants	305	333	326	266
Number of Resorptions[a]				
Early	24/14	22/15	26/13	15/11
Middle	0/0	3/3	0/0	8/6[b]
Late	0/0	0/0	0/0	5/4
Fetal Weight, g[c]	4.50 ± 0.49	4.33 ± 0.52	4.33 ± 0.53	3.55 ± 0.38
Crown-Rump Length, mm	40 ± 2	39 ± 2	39 ± 2	36 ± 1
Placental Weight, g	0.49 ± 0.06	0.49 ± 0.10	0.46 ± 0.05	0.34 ± 0.06
Number of Fetuses Examined[a]	280/23	308/25	300/25	238/23
Reduced Ossification	3/2	7/4	8/6	28/10
Small Lungs	0/0	0/0	0/0	20/8
Cleft Palate	0/0	0/0	0/0	10/2
Dilated Ureter	17/11	31/18	17/9	0/0
Maternal Measures				
Body Weight at Sacrifice, g	306 ± 27	312 ± 33	302 ± 29	271 ± 25
Extragestational Body Weight, g[d]	231 ± 15	240 ± 22	234 ± 15	220 ± 18
Tissue Weights[e]				
Lung	0.42 ± 0.02	0.43 ± 0.04	0.44 ± 0.04	0.50 ± 0.06
Spleen	0.18 ± 0.02	0.22 ± 0.03	0.20 ± 0.03	0.22 ± 0.03
Thymus	0.095 ± 0.03	0.095 ± 0.03	0.082 ± 0.03	0.040 ± 0.01

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[a] Data are expressed as number of fetuses/number of litters.

[b] Statistically significant differences from control group are underlined.

[c] Data reported as the mean of litter means ± standard deviation.

[d] Sacrifice body weight minus weight of uterus with products of conception

[e] Expressed as percent of extragestational body weight

The results of this experiment are summarized in Table 1. There was a statistically significant increase (P = 0.01) in the incidence of midgestational deaths of the conceptuses in the group exposed to the highest concentration of HD. Incidence of late resorptions was also increased (P = 0.055) relative to the control group. Since the periods of middle and late resorptions (combined) approximately correspond to the gestational period of HD-exposure, the increased incidence of resorptions appears attributable to exposure.

The sizes of components of the fetoplacental unit (crown-rump length, fetal weight, and placental weight) were smaller in the high-exposure group than in the control group (P = 0.01), but were not significantly affected in the middle- and low-exposure groups. Ossification was significantly reduced in the calvarium and vertebral column of the fetuses of the high-dose group.

Morphologic changes were found only in fetuses of pregnant rats exposed to the highest concentration of HD. The most prevalent indicator of dysmorphology was "small lungs," a reduction in size which was readily apparent on gross examination at low-power magnification. The incidence of this defect was significantly (P = 0.01) increased in the high-exposure group; small lungs were not observed in the middle- and low-exposure groups. Cleft palates were also observed in the high-exposure group, but the observed increase in incidence of affected litters was not statistically significant. Approximately 6% of the fetuses, distributed over 48% of the litters in the control group, had ureters that appeared larger than normal in diameter, which is consistent with our past experience with this strain of animal. This incidence did not change significantly in the low- and middle-exposure groups. Ureters with an appearance consistent with other strains (i.e., not dilated) were observed in fetuses of dams from the high-exposure group. This difference was statistically significant, but its biological import is unclear at this time.

Maternal body weight at sacrifice for animals in the high-exposure group was significantly lower than that of controls (Table 1). This decrease was statistically significant in two of the three replicates, and also when the three replicates were combined, suggesting possible maternal toxicity. Reduction in maternal body weight observed at sacrifice was primarily due to the indicated decrease in the size of the products of conception, since analysis of the extragestational body weights showed that there were no significant differences between the control and the exposed groups.

The weights of maternal tissues at sacrifice provided another basis for evaluating maternal toxicity (Table 1). Thymus weight was significantly decreased in the high-exposure group; a dose-dependent response was suggested by the slight, but not statistically significant, reduction in thymus weight in the middle-exposure group. Lungs and spleen were significantly heavier in the high group. Weights of liver, kidney, and adrenal glands were not different from those of controls.

Our data, therefore, indicate that inhalation exposure to HD produced effects in the fetus at an exposure level that did not influence maternal body mass. It is not clear if alterations in the weights of the maternal lung, spleen, and thymus by the high dose reflect maternal toxicity or are nonspecific responses to stresses.

Oral Administration

Most elements of the design and procedures used in the experiment to define dose-response relationships and estimate minimal effective doses after oral administration are best presented in comparison to the earlier survey experiments, since this later experiment did not attempt to replicate materials or protocols. Exposure to HD in both studies was by daily gavage during the period from 12 through 16 dg. The two lower doses from the survey experiment were repeated as the high doses in the present study. The HD was from the same batch and the rats were of the same stock (Charles River, CD) as those used in the inhalation study described above; both were different from those used for the survey experiment, which used HD from a different batch, and Wistar (Hilltop) rats. Moreover, in the present study, the HD was diluted in milk (rather than undiluted), the controls were gavaged with milk (instead of corn oil), and 6-aminonicotinamide (instead of Aroclor) was used as the positive control material. Broader maternal and fetoplacental evaluations, similar to those described for the inhalation studies, were employed, and sacrifice was at 20 (rather than 21) dg. Furthermore, because of the increased incidence of "small lungs," as reported above for the inhalation studies and noted at the higher doses in the survey experiment, fetal lungs were removed and weighed at sacrifice.

The experiment was performed in three blocks, the first two of which were essentially identical. Because of the unacceptably high mortality at the high dose (Table 2) and the shape of the fetal dose-response curves, the high dose was omitted from the third block and an additional intermediate dose was included. The resulting range of dose groups and group sizes, and some representative results to date, are summarized in Table 2. Detailed teratological evaluations of the heads and skeletons, as well as comprehensive data tabulations and analyses, are currently being completed.

Intrauterine mortality increased as a function of dose beyond the 0.14-g/kg level, although individual incidences were not significantly increased above control levels (on pairwise comparisons) until the 0.37-g/kg level. The 0.14-g/kg dose appeared to be the lowest which increased the incidence of fetuses with small lungs, both on an individual and a litter basis. There was a trend toward decreasing fetal weight with increasing dose, although the mean values did not differ from those of controls, on pairwise comparison, except at the highest level (Figure 1). Fetal lung weight also showed a similar dose-dependent decrease and was also significantly affected at lower doses (0.18 g/kg). As a result, fetal lung weight, expressed as a percentage of fetal weight (Figure 1), showed a progressive decrease with increasing dose at all levels. Pairwise comparison showed a significant difference in this measure at the 0.14-g/kg level.

A variety of other malformations, e.g., cleft palate, were noted on gross teratologic evaluation. These measures, await-

TABLE 2. Effects of Intragastric Intubation of Pregnant Rats with SRC-II Heavy Distillate from 12-16 Days of Gestation.

	Dose, g/kg					
	0	0.09	0.14	0.18	0.37	0.74
Number of Litters Examined	27	20	20	26	21	3
Intrauterine Mortality, %[a]	5.1 ± 1.4	3.2 ± 1.3	9.3 ± 2.6	10.7 ± 2.3[b]	16.6 ± 3.6	61.7 ± 31.1
No. Live Pups Examined	271	216	211	258	164	3
Incidence of "Small Lungs"[c]	0/9	1/1	24/4	36/9	91/18	3/2
Maternal Mortality, Fraction; % in parentheses	0/35 (0)	0/32 (0)	0/25 (0)	0/26 (0)	0/27 (0)	10/16 (67)
Body Weight at 20 dg	334 ± 7	321 ± 8	332 ± 8	306 ± 6	281 ± 6	259 ± 8
Extragestational Weight[d]	280 ± 6	273 ± 6	283 ± 7	263 ± 4	247 ± 4	251 ± 12
Organ Weights[e]						
Spleen	0.22 ± 0.005	0.24 ± 0.025	0.22 ± 0.006	0.23 ± 0.008	0.25 ± 0.007	0.21 ± 0.005
Thymus	0.12 ± 0.007	0.08 ± 0.004	0.07 ± 0.005	0.07 ± 0.003	0.06 ± 0.003	0.05 ± 0.007

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[a] Data reported as the mean of litter means ± standard error.

[b] Statistically significant differences from control group are underlined.

[c] Data are expressed as number of fetuses/number of litters.

[d] Sacrifice body weight minus weight of the uterus with products of conception

[e] Expressed as percent of extragestational body weight

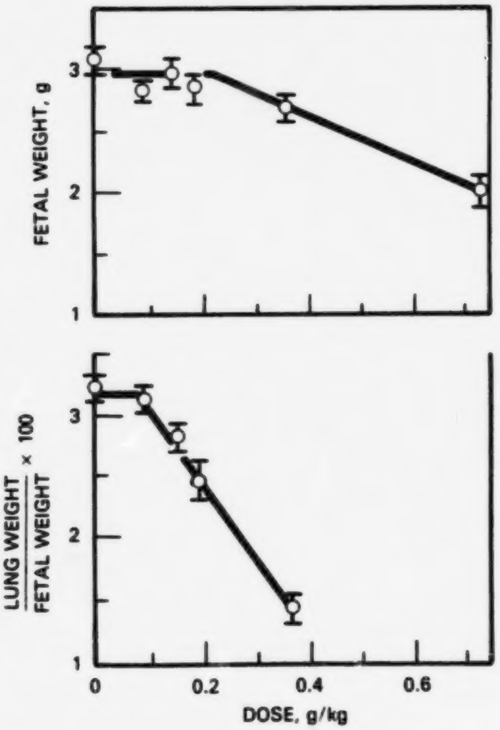

FIGURE 1. Effect of Gastric Intubation of SRC-II Heavy Distillate on Fetal Rat Weight and Relative Lung (Lung/Body) Weight (Mean ± SE).

ing verification on detailed evaluation, have not yet been tabulated, so that their relationship to dose is not established. Nonetheless, it appears that the minimal dose which will produce embryotoxicity and/or teratisms will lie in the approximate range of 0.14-0.18 g/kg.

On the other hand, 0.18 g/kg was the lowest dose at which there was even a suggestion of maternal toxicity (as indicated by body weight at 20-dg sacrifice). The embryolethality and fetal weight reduction observed at this level are reflected by the fact that the corresponding extragestational weight was less markedly reduced below the control level, and that it was only at the 0.37-g/kg level that a statistically significant decrease is evident. Maternal organ weights, expressed as percent of extragestational weight, showed a trend toward increase in hepatic weight with dose similar to that seen after inhalation exposure. Thymus weight was reduced in all exposed groups but did not show a dose-response relationship. (Lung weight was not measured.) This result makes it less clear as to whether these tissue weight changes reflect maternal toxicity or a nonspecific response to stress associated with exposure to an unpalatable mixture. On the basis of body weight alone, the data suggest that the maternally toxic level lies between 0.18 and 0.37 g/kg, and that embryotoxicity is produced at levels below those which are maternally toxic.

• Inhalation Hazard to Coal Miners

Principal Investigator: M. T. Karagianes

Other Investigators: P. J. Mihalko, R. F. Palmer, R. A. Miller,and D. B. Carr

Acceleration in coal mining activities to satisfy present and future energy needs may be constrained by potentially severe health hazards. Coal workers' pneumoconiosis (CWP), a chronic debilitating disease associated with coal mine dust, is one known entity; additional inhalation hazards may occur with the proposed extensive use of diesel engines in coal mines.

The objectives of this project are to evaluate the biological effects of specific mine air contaminants in experimental animals, and to study the progression of chronic respiratory pathologic changes that occur with coal dust and/or diesel exhaust inhalation.

In order to determine if impaired pulmonary function is an additional risk factor in the occupational exposure of workers to mine-air contaminants, an elastase-induced obstructive lung lesion was produced in Sprague-Dawley rats prior to their exposure to coal dust and diesel engine exhaust (Annual Report, 1980). These impaired animals, along with groups of normal rats, were exposed, separately and in combination, by whole-body inhalation, for 10 weeks, to unscrubbed exhaust from an inefficient diesel engine and ground Pittsburgh Montour #4 coal dust (Table 1). Lung volumes and forced-ventilatory parameters were measured prior to and following exposures. The animals were sacrificed after postexposure testing, and their respiratory tracts were examined by light microscopy.

TABLE 1. Conditions for Inhalation Exposure of Normal and Elastase-Impaired Rats to Coal Dust and Diesel Engine Exhaust.

Chamber	Number of Rats	Conditions[(a)]
I	10 Normal 10 Elastase	Unscrubbed, Diesel Exhaust 50 ppm CO, <10 ppm NO_x, ~27 mg/m^3 Soot, MMAD[(b)] = 0.33 μm, GSD[(c)] = 3.67
II	10 Normal 11 Elastase	Unscrubbed, Diesel Exhaust 50 ppm CO, 10 ppm NO_x, Pittsburgh Montour #4 Coal Dust[(d)] Total Particulates ~37 mg/m^3, MMAD = 0.50 μm, GSD = 5.02
III	12 Normal 13 Elastase	Pittsburgh Montour #4 Coal Dust Total Particulate ~20 mg/m^3, MMAD = 2.31 μm, GSD = 1.99
IV	13 Normal 11 Elastase	Filtered Room Air

(a) 6 hr/day, 5 days/week

(b) Mass Median Aerodynamic Area, determined by Andersen cascade impactor techniques

(c) Geometric Standard Deviation

(d) Ground for 14 hr under a 100% nitrogen atmosphere

Elastase-impaired animals demonstrated significantly increased lung volumes and reduced forced-expiratory flow rates when compared to normal animals, indicating the presence of an obstructive pulmonary lesion prior to inhalation exposures ($P < 0.01$, *t*-test). Pre- and postexposure lung function measurements were compared, with respect to exposure group and elastase-impaired or normal status, by a two-factor analysis of variance with repeated measures (Table 2). The results indicated progressive degradation of lung function in elastase-impaired animals regardless of inhalation exposure group. Both normal and elastase-impaired animals exposed to diesel exhaust, alone and in combination with coal dust, demonstrated significantly increased functional residual capacities ($P < 0.05$ Tukey range test, Table 3).

Histopathologic observations confirmed the presence of mild to moderate panlobular emphysema in elastase-impaired animals. Both normal and elastase-impaired animals exposed to coal dust alone had mild focal accumulations of coal-dust-laden macrophages perivascularly, around alveoli, distal airways, and in bronchus-associated lymphoid tissue of larger airways. Minimal alveolar epithelial hyperplasia was apparent. Animals exposed to diesel exhaust, alone and in combination with coal dust, showed similarly located exposure-related lesions of greater intensity. Hyperplasia of alveolar epithelium was moderate in normal animals of those groups, and slight in elastase-impaired animals.

Significant increases in functional residual capacity and alveolar epithelial hyperplasia resulted from the exposure of normal and elastase-impaired rats to diesel engine exhaust, alone and in combination with coal dust, but not from exposure to coal dust alone. Elastase-impaired rats appeared to have less-intense alveolar epithelial hyperplastic

TABLE 2. Lung Function Measurements in Normal and Elastase-Impaired Rats Before and After Inhalation Exposure to Coal Dust and Diesel Engine Exhaust. (Values shown in boldface are statistically significant.)

Parameter		Diesel Exhaust			Diesel + Coal Dust			Coal Dust			Filtered Room Air			Two Way Anova, Repeated Measures				
		N	Pre	Post	N	Pre	Post	N	Pre	Post	N	Pre	Post	Degrees of Freedom	Mean Square Error	P Elastase-Treatment Interaction	P Treatment Effects	P Elastase Effects
Body Weight, g	Normal	8	704	730	9	666	677	11	660	682	14	667	689	75	954.1	0.925	0.482	0.224
	Elastase	8	669	718	10	663	681	13	649	683	10	655	683					
Respiratory Rate, breaths/min	Normal	7	113	99	9	119	88	11	100	81	14	100	99	74	534	0.410	0.517	0.518
	Elastase	8	92	94	10	88	82	13	87	91	10	89	84					
Tidal Volume, cm^3	Normal	8	2.7	2.0	9	2.2	1.8	11	2.5	2.0	14	2.7	2.1	70	0.218	0.497	0.686	0.765
	Elastase	7	2.5	2.3	9	2.9	2.3	11	2.7	2.1	9	2.9	2.3					
Vital Capacity, cm^3	Normal	8	18.4	17.1	9	18.0	14.6	11	17.2	16.4	14	18.7	16.4	75	3.439	0.200	0.390	**0.047**
	Elastase	8	20.1	20.1	10	19.3	18.8	13	19.4	18.1	10	20.5	19.2					
Inspiratory Reserve Capacity, cm^3	Normal	7	16.7	13.7	9	15.1	10.6	11	15.4	12.2	14	15.2	12.6	74	8.513	0.902	0.281	0.156
	Elastase	8	17.0	15.0	10	17.7	14.4	13	16.2	14.0	10	16.7	14.2					
Functional Residual Capacity, cm^3	Normal	6	5.5	7.5	8	5.8	6.9	10	5.6	5.6	14	5.7	5.0	63	1.464	0.526	**0.005**	0.740
	Elastase	6	7.0	7.8	8	7.0	8.4	12	7.7	7.4	7	6.8	6.7					
Expiratory Reserve Volume, cm^3	Normal	7	1.5	2.3	7	1.8	2.5	8	2.1	2.1	11	2.1	2.1	63	1.039	0.397	1.437	**0.086**
	Elastase	8	1.8	3.4	8	2.3	2.1	13	2.9	2.1	9	3.0	3.2					
Total Lung Capacity, cm^3	Normal	6	23.2	22.9	6	22.7	19.4	8	21.0	19.5	8	21.0	19.5	54	5.19	0.222	0.415	0.483
	Elastase	6	25.9	24.3	7	23.9	24.3	12	24.7	22.6	6	25.1	22.5					
Residual Volume, cm^3	Normal	6	3.8	5.2	6	4.2	4.8	8	3.5	3.4	11	3.5	2.9	54	2.77	0.616	0.363	0.122
	Elastase	6	5.6	4.5	7	5.4	6.0	12	5.5	4.6	6	4.9	3.8					
Time Constant of Passive Expiration, sec	Normal	7	0.376	0.473	9	0.303	0.530	11	0.324	0.383	14	0.324	0.383	74	0.018	0.078	0.835	0.661
	Elastase	8	0.371	0.472	10	0.376	0.466	13	0.294	0.531	10	0.346	0.497					
Maximal Experimental Flow Rate, cm$^3 \cdot$ sec^{-1}	Normal	8	156.8	145.7	9	160.2	160.1	15	153.2	163.8	14	149.4	152.4	75	842.9	0.5988	0.147	**0.028**
	Elastase	8	153.9	138.9	10	170.8	141.4	13	142.9	140.7	10	155.8	146.3					
Expiratory Flow at 80% Vital Capacity, cm$^3 \cdot$ sec^{-1}	Normal	8	106.1	117.2	9	123.8	119.3	11	121.9	119.8	14	119.5	122.9	75	271.3	0.359	0.448	0.235
	Elastase	8	99.3	99.2	10	105.7	110.4	13	103.3	93.9	10	111.3	98.9					
Expiratory Flow at 60% Vital Capacity, cm$^3 \cdot$ sec^{-1}	Normal	8	73.6	77.5	9	82.8	73.7	11	72.1	76.3	14	74.1	69.9	75	160.1	0.300	0.458	0.129
	Elastase	8	62.1	55.6	10	62.0	60.1	13	60.6	52.9	10	65.9	52.1					
Expiratory Flow at 50% Vital Capacity, cm$^3 \cdot$ sec^{-1}	Normal	8	51.6	46.6	9	62.6	52.2	11	53.8	52.7	14	53.9	48.0	75	74.63	0.413	0.548	0.871
	Elastase	8	44.5	38.7	10	42.7	39.4	13	42.5	37.4	10	45.2	35.1					
Expiratory Flow at 10% Vital Capacity, cm$^3 \cdot$ sec^{-1}	Normal	8	8.4	5.3	9	11.6	4.4	11	9.7	4.5	14	8.2	4.5	75	8.21	0.230	0.594	0.067
	Elastase	8	9.1	5.5	10	7.5	4.4	13	6.8	4.5	10	8.2	4.5					

responses in the diesel exhaust groups. The impaired animals in all groups, including controls, that were exposed only to filtered room air had progressive degradation in lung function, with time.

The elastase-impaired rat model of pulmonary emphysema did not clearly demonstrate any synergistic effects between impaired lung function and exposure to coal-mine air contaminants.

TABLE 3. Comparison[a] of Functional Residual Capacity of Normal and Elastase-Impaired Rats After Inhalation Exposure to Coal Dust and Diesel Exhaust.

	Diesel	Diesel/ Coal Dust	Coal Dust	Control
Mean Change (before-after)	1.39	1.26	−0.11	0.51
N:	12	16	22	21
Mean Square: 2.93				
Degrees of Freedom: 63				
$P < 0.05$				

[a] Tukey range test

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• Factors Influencing Cross-Placental Transfer and Teratogenicity of Metallic Pollutants

Principal Investigator: P. L. Hackett

Technical Assistance: A. J. Clary, R. L. Music, and S. A. Watson

Results from studies involving lead exposures (intravenously, by inhalation, and via intragastric intubation) at representative gestational ages (9, 15, and 18 days of gestation; dg) have shown that Pb mass affects metabolism. The rapid uptake of Pb into the fetoplacental unit (FPU), which was dependent upon the rate and extent of maternal absorption, was more pronounced in later stages of gestation. Just before parturition, Pb burdens were concentrated in the fetus, rather than in membranes or placenta, irrespective of stage of development at exposure. Embryotoxicity and teratogenesis were observed at dose levels producing overt maternal toxicity and were directly related to the time of administration.

Studies are in progress to determine potentially embryotoxic effects of vanadium in rats. Both dose level and stage of gestation (9, 15, or 19 dg) significantly influenced V distribution in maternal tissues and V accumulation in the FPU. Parenteral administration of high doses of V at 15 dg produced maximal intrauterine mortality even though V deposition was highest in the 19-dg FPU. The major fraction of the V burden in the FPU was in the membranes. In animals injected at 9 or 15 dg, for whom intrauterine exposure is extended, fetal accumulation was independent of dose. For shorter exposure periods resulting from injection at 19 dg, fetal V burdens were proportional to the dose.

Coal and crude petroleum oils contain a variety of metallic impurities, among them, vanadium, an essential trace mineral, which is toxic when present at elevated levels in adults. High, localized concentrations have been reported in the placenta, visceral yolk sac, and in fetal bone after intravenous (IV) injection in near-term mice. No studies have been reported on the potential embryotoxic effects of vanadium, or on its distribution at earlier gestational ages. Accordingly, we have conducted screening experiments to determine the existence of such effects, and to obtain preliminary data on placental transfer and distribution within the fetoplacental unit (FPU).

We have compared ^{48}V distribution following intraperitoneal (IP) administration of "tracer" or "carrier" solutions to pregnant rats at 9, 15 or 19 dg. Tracer solutions consisted of ^{48}V in saline, adjusted to pH 7. Carrier solutions contained 5 mg V/kg body weight, as $Na_3VO_4 \cdot 5H_2O$, in addition to the ^{48}V and vehicle. Since the pregnant uterus may comprise as much as 20% of maternal body weight in the more advanced stages of pregnancy, dose volumes were calculated for individual rats on the basis of their 9-dg weight, in order to avoid undue toxicity and mortality of animals injected at 15 or 19 dg. These rats were sacrificed at 1.5, 6 and 24 hr after injection, and at 20 dg, to determine ^{48}V activities in maternal and fetal tissues.

When the conceptus was examined for viability at each sacrifice time, embryotoxicity was higher in rats injected with carrier at 15 dg than at 9 dg or 19 dg. It was also evident that the toxic effects were manifested soon after V administration (Table 1). These data confirm results from an earlier screening study in which

TABLE 1. Intrauterine Mortality in Rats Injected with Vanadium (%, Mean ± SE).

Day of Gestation at Injection	Time After Injection	Tracer[a]		Carrier[b]	
9	1.5 hr	(75/7)[c]	0	(27/2)[c]	0
	6 hr	(57/7)	0	(43/3)	0
	24 hr	(48/5)	0	(42/3)	0
	11 day	(81/6)	1.1 ± 1.1	(74/6)	4.2 ± 2.9
15	1.5 hr	(86/6)	5.8 ± 2.2	(70/7)	24.1 ± 13.2
	6 hr	(83/6)	8.0 ± 1.9	(51/5)	26.9 ± 18.5
	24 hr	(85/7)	10.4 ± 4.6	(81/6)	45.7 ± 18.5
	5 day	(89/7)	6.5 ± 3.5	(51/4)	65.8 ± 17.9
19	1.5 hr	(79/7)	3.2 ± 1.5	(39/4)	4.3 ± 2.4
	6 hr	(51/4)	1.7 ± 1.7		-
	24 hr	(82/6)	7.8 ± 3.0	(57/4)	12.5 ± 4.5

[a] ^{48}V

[b] ^{48}V + 5 mg V/kg body weight

[c] Number fetal implants/number of litters

we found maximal embryotoxicity at 14-15 dg following injection of 5 mg V/kg.

The stage of gestation at the time of ^{48}V adminstration, and the dose level, significantly influenced V distribution in maternal tissues (Table 2). In general, ^{48}V values were higher in skeletons of carrier-dosed rats than in those of the tracer group. Muscle and kidney appeared to accumulate and retain more of the tracer dose. Tissue values for animals injected at 19 dg tended to be lower than those of rats injected at 9 or 15 dg. Plasma concentrations and plasma clearance rates of ^{48}V resembled patterns observed for soft tissues (Figure 1). Clearance of ^{48}V was faster in carrier- than in tracer-dosed animals, and plasma concentrations were lowest 24 hr after injection of rats in the most advanced stages of pregnancy.

The percentage of the ^{48}V dose appearing in the FPU was dose- and age-dependent (Figure 2). Larger fractions of ^{48}V were observed in the more-developed, 19-dg FPU, and values for carrier-exposed FPUs were lower than those of the tracer group. However, by 5 or 11 days after injection, FPUs in both dose groups contained nearly equal fractions of the ^{48}V dose.

At 1.5 hr after injection, the 15-dg and 19-dg placenta contained the largest fraction of the ^{48}V FPU burden (Figure 3). Values for carrier-exposed placentas tended to be higher, and membrane values lower, than those of the tracer group. Placental clearance was rapid, therefore acquisition of the major ^{48}V fraction by the membrane had occurred by 24 hr after injection. Fetal values for 15-dg FPUs were generally lower than membrane levels and tended to be independent of vanadium dose level. Percentages in the fetus increased during the 5-day uterine exposure. Comparable fetal deposition was observed for the 9-dg tracer- and carrier-dosed animals whose 20-dg values were 21% and 18%, respectively. Deposition was dose-dependent in older, 19-dg FPUs, where observed values for carrier-exposed fetuses were higher and membrane values lower than those of the tracer-dosed rats.

TABLE 2. The Effect of Pregnancy and Dose Level on ^{48}V Distribution in Maternal Tissues at 24 hr After Administration (Mean ± SE).

Tissue	Dose	Days of Gestation at Injection		
		9	15	19
Skeleton	Tracer(e)	13.7 ± 1.18(b,c)	11.6 ± 0.91(c)	7.70 ± 0.98(d)
	Carrier(f)	24.0 ± 2.38(a,b)	23.7 ± 2.52(a)	15.0 ± 0.92(a,b,c)
Muscle	Tracer	5.04 ± 0.44(a,b)	5.59 ± 0.30(a)	3.34 ± 0.28(b)
	Carrier	2.27 ± 0.35(b,c)	2.48 ± 0.41(b,c)	1.26 ± 0.07(c)
Liver	Tracer	2.28 ± 0.40	3.27 ± 0.21	3.27 ± 0.19
	Carrier	2.09 ± 1.38	2.97 ± 0.63	1.17 ± 0.10
Kidney	Tracer	2.91 ± 0.24(a)	3.15 ± 0.13(a)	2.76 ± 0.43(a)
	Carrier	2.39 ± 0.41(a,b)	2.27 ± 0.35(a)	0.90 ± 0.11(b)

(a-d) Values for each tissue that do not share a common letter are significantly different ($P < 0.05$).

(e) ^{48}V

(f) ^{48}V + 5 mg V/kg body weight

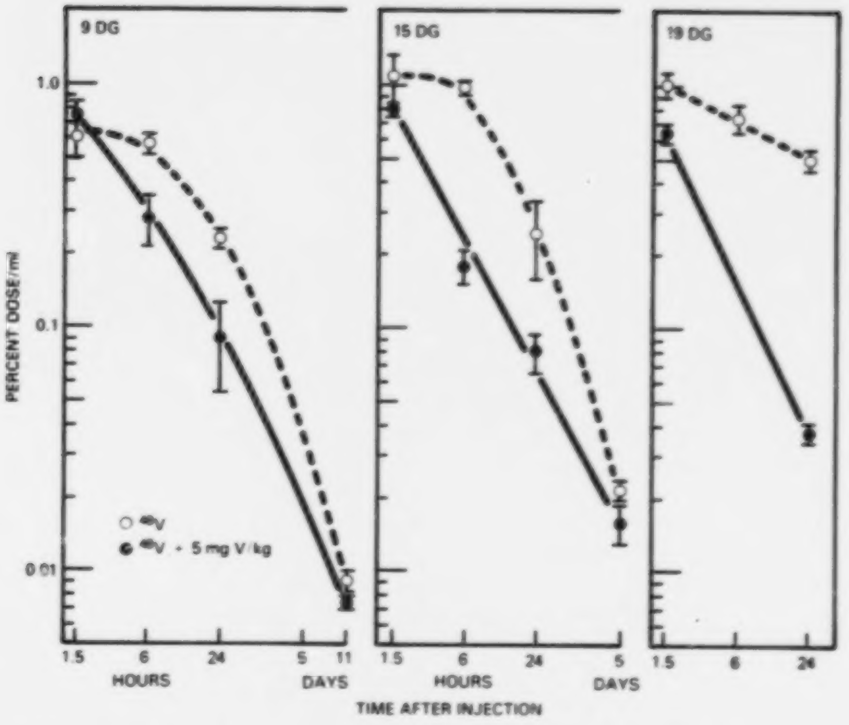

FIGURE 1. ^{48}V Plasma Concentrations in Rats Injected at 9, 15 or 19 Days of Gestation.

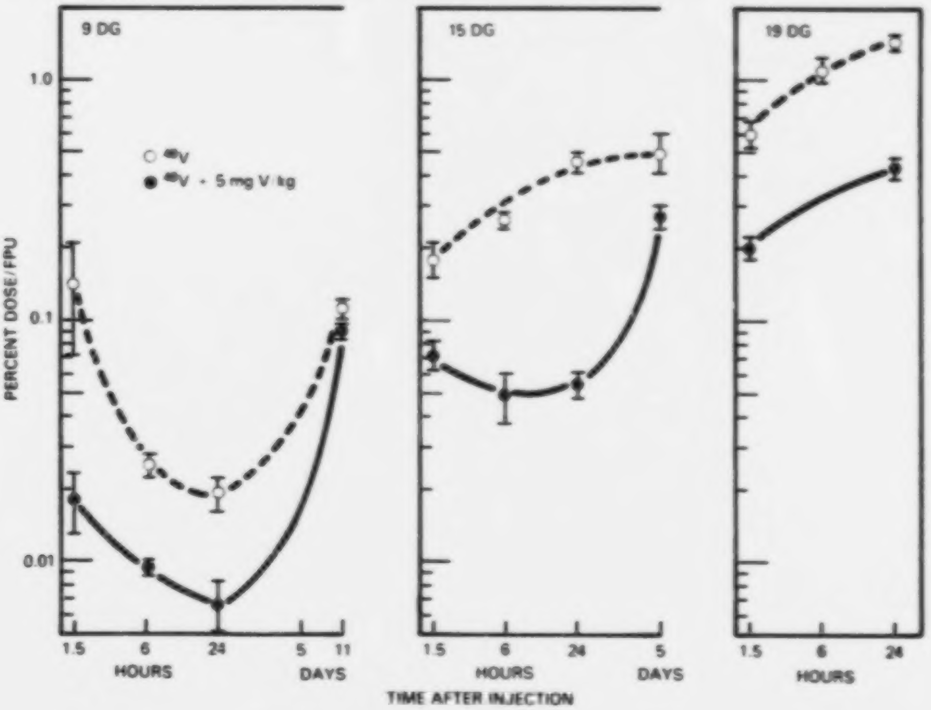

FIGURE 2. Percentage of ^{48}V Dose in Fetoplacental Units of Rats Injected at 9, 15 or 19 Days of Gestation.

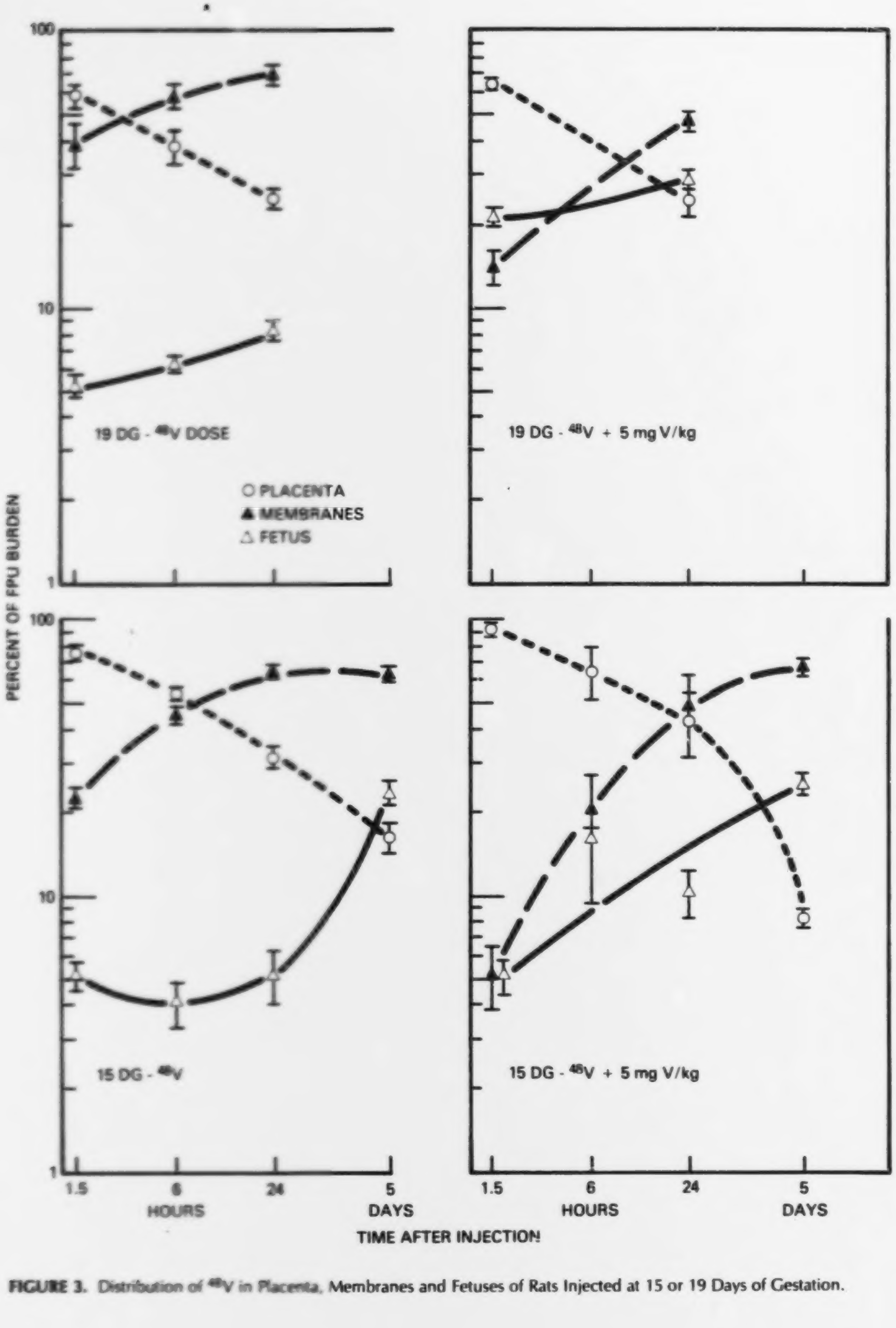

FIGURE 3. Distribution of ^{48}V in Placenta, Membranes and Fetuses of Rats Injected at 15 or 19 Days of Gestation.

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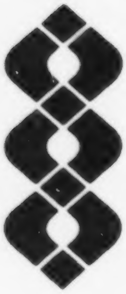

Conservation

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CONSERVATION

This section reports on progress made on projects concerned with potential health effects of exposure to static and 60-Hz electric fields from high-voltage transmission systems. They seek to determine whether such fields can produce genetic effects in biological systems and develop a dosimetric basis for extrapolating effects from laboratory studies on animals and cellular system to humans.

These projects are part of the Pacific Northwest Laboratory bioelectromagnetics program and are complemented by ongoing 60-Hz electric field studies in laboratory rodents, sponsored by the DOE Office of Electric Energy Systems, and in miniature swine, sponsored by the Electric Power Research Institute.

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• Mutagenic Effects of Electric Fields

Principal Investigator: F. P. Hungate

Other Investigators: L. K. Fritz and G. L. Williams

Data have been inconclusive from experiments designed to ascertain if exposure to electric fields causes biological effects in mammals and have not yet led to identifying mechanisms by which effects might be produced. Even when animals are exposed to extremely high-strength fields (100 kV/m), the field within the body is low (mV/m) because body fluids are highly conductive. In vitro exposure of cells in suspension, however, allows measurement of cellular effects in field strengths much higher than those to which whole animals can be exposed. The present study is designed to examine simple cell systems for evidence as to whether electric fields affect the function of the genetic system.

At this stage, there is no evidence that even the highest field strengths affect the normal cell mutation frequency; however, gene expression is affected, as shown by increased frequency of induced phage.

A new system for exposing cells in culture to electric fields was described in last year's Annual Report. To protect cells from potentially toxic materials produced at the electrodes, this system uses agar plugs having the same ionic composition as the culture medium. We have now made three revisions to the system: 1) a side arm has been installed to provide easier accessibility and to eliminate the problem of electrode/agar-plug separation, 2) graphite has been substituted for platinum as the electrode material, and 3) the units are now mounted on a wrist-action shaker to maintain the cells in uniform suspension. (The shaker introduces less than 1% variance in the exposure field.)

With this new exposure system, the Ames Salmonella genetic test strains TA98 and TA100 were evaluated for possible induction of mutations, using 60-Hz fields up to 120 V/m for as long as 24 hr. No increased frequency of mutations to prototrophy was observed in either strain. Tests were conducted both with and without activating S9 enzymes present in the medium during exposures or in the plating medium used for assaying the response.

In previous testing we observed an apparently increased loss of plasmids carried in Escherichia coli due to exposure to the electric field. Retesting of antibiotic-resistant plasmids RGK, R600 and RPj4 showed no differences in rate of plasmid loss between unexposed cells and those exposed to fields up to 150 V/m (60 Hz) for as long as 24 hr.

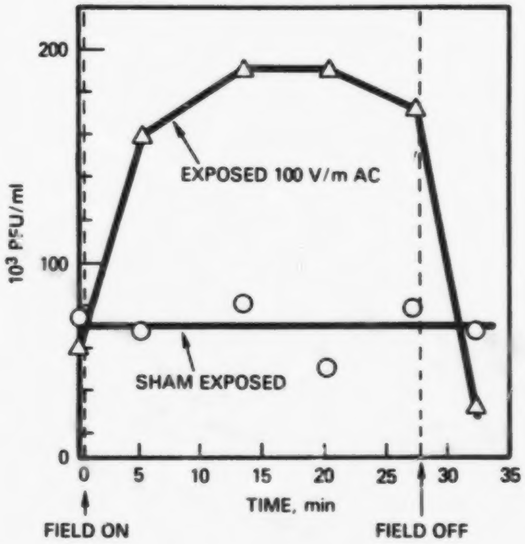

FIGURE 1. Effects of a 60-Hz Electric Field on Phage Induction as Measured by Free-Phage Plaque Produced. (PFU, plaque-forming units).

A small subpopulation of cells carrying phage (less than 10% of the total) were induced to lyse in the presence of the 60-Hz field (Figure 1). Induction began immediately with energizing of the field, becoming irreversibly established after 5 min. This is shown by the fact that induced cells continued to burst through the lytic cycle, even when the field was turned off after 5 min. However, discontinuing exposure before 5 min resulted in a return of induced cells to dormancy. (Induced λ-phage are known to begin DNA synthesis at about 5 min.) Once irreversibly induced, cells proceeded through a single burst cycle, but subsequent burst cycles were not induced. Thus there was no continuing increase of inductions with longer exposure regimes. The data suggest that sensitivity to the field is a physiologic condition, perhaps aberrant, and that all susceptible cells in a population are induced and burst in the initial induction cycle.

Mutant strains of cells with reduced (or completely absent) capacity for prophage induction by electric fields have been isolated, and their induction loss was genetically maintained. We have not yet succeeded in isolating strains having more than 10% of the population susceptible to induction.

Since available data suggest that the effect of ac fields on genetic systems is mediated through processes other than mutation, we are now directing attention toward systems capable of examining processes other than mutation. Preliminary research suggests that electric-field exposure may affect uptake, processing and/or recombinations of infective, transforming, or transfecting DNA. Studies on the effect of electric fields on the process of genetic transformation and recombination of DNA are continuing.

• Electric Field Dosimetry

Principal Investigator: W. T. Kaune

Other Investigators: C. K. Chou* and A. W. Guy

Technical Assistance: W. C. Forsythe, M. McCumber *, and G. Y. Yang *

Data on electric fields and current densities inside the bodies of animals and humans are needed to relate exposure levels used in the laboratory with experimental animals to those experienced by humans from electric-power generation, transmission, and distribution systems. We report here preliminary measurements in the bodies of homogeneous models of humans, rats, and swine exposed to 1-kV/m, 60-Hz electric fields.

A number of projects are underway to investigate whether electric fields can produce biological effects in laboratory animals. However, before data from these projects can be used for the assessment of possible risks to humans exposed to power-frequency electric fields, dosimetric characterization for humans and animals is needed. The purpose of this project is to determine the electric fields and current densities induced inside the bodies of humans and animals exposed to such electric fields.

We have pursued two different methods of measurement. The first, being carried out at PNL, uses saline-filled conducting models exposed directly to 60-Hz electric fields. The second, being developed at the Bioelectromagnetic Research Laboratory, University of Washington, uses radiofrequency exposure and thermography to obtain data which are then extrapolated to 60 Hz.

Measurement of Induced 60-Hz Current Densities in Models of Humans and Animals

In our 1980 Annual Report, we described the first year's progress in the following areas: 1) construction of saline-filled hemispherical and hemispheroidal models; 2) construction and calibration of probes suitable for the measurement of fields in saline-filled models; 3) construction and calibration of electronics to process the field-probe signals; and 4) tests with hemispherical and hemispheroidal models, exposed to power-frequency electric fields, which demonstrated the feasibility and accuracy of the measuring technique.

In the past year, progress has been made in the following areas: 1) construction of saline-filled models of humans, rats, and swine; and 2) measurement of induced fields in grounded human, rat and swine models.

Conducting Models

Conducting models were made by filling hollow styrofoam molds (which do not significantly perturb an applied electric field) with saline solution. The styrofoam molds are prepared in halves, corresponding to the two halves of a human body divided along the frontal plane; or to the two halves of a rat or pig, divided along a sagittal plane. A field probe was positioned inside the mold, and the two halves were reassembled, using paraffin as a glue/sealant. Copper pads, attached to the bottom of each foot, were instrumented so that the current passing through each foot could be measured and controlled.

Induced-Current Measurements

Our data are presented in terms of induced current density, rather than induced electric field strength, because current density is independent of the particular conductivity of the saline-filled model. Current density can, therefore, be more easily extrapolated to other models or to live animals. (Current density is the current crossing a unit area, oriented perpendicularly to the direction of current flow.) Electric field strength can be obtained by dividing the current density by the conductivity of the body.

Vertical and horizontal current densities measured in the frontal plane of the human body are shown in Figure 1. The directions of the horizontal components of current density represent an estimate. (These directions will be measured in the future.) The model was grounded so that equal currents passed through both feet.

Measurements were also made with the total induced current passing to ground through

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*University of Washington

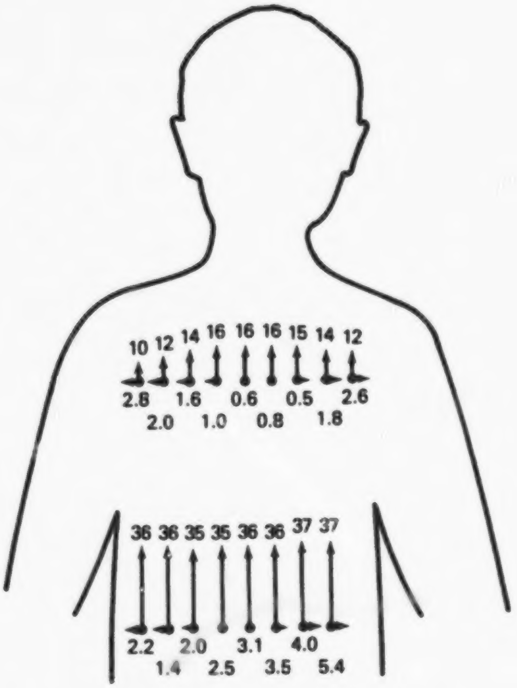

FIGURE 1. Measured Current Densities (nA/cm^2) in the Midline Frontal Plane of a Conducting Human Model Exposed to a 1-kV/m, 60-Hz Electric Field. Both feet of the model were grounded, with equal currents passing through them.

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only one foot. Although current densities measured in the chest were not affected, vertical current densities in the abdomen above the grounded foot were about 25% greater than over the ungrounded foot.

Figures 2 (rat) and 3 (swine) show vertical and horizontal current densities measured in the sagittal planes of conducting models. (The direction of the horizontal current densities were not measured.) For these measurements, the currents passing to ground through all four feet were equalized. When only the front or rear feet were grounded, the horizontal current density increased (by as much as a factor of 5) over that when all four feet were grounded. Vertical current densities were lower above the ungrounded feet and higher (by as much as a factor of 2) over the grounded feet.

Comparison of human and animal data given in Figures 1 through 3 leads to the following conclusions: 1) induced currents in the torso of a grounded human exposed to a 1-kV/m, power-frequency electric field are 10-20 times greater than the currents induced by the same electric field in grounded rats and pigs; and 2) induced currents in the torso of a pig are about twice those induced in the torso of a rat when both species are exposed to the same electric field.

The conductivities of human, swine and rat tissues are approximately equal; therefore these conclusions are also valid for induced electric fields.

Determination of Electric Current Distributions in Man and Miniature Swine Exposed to Uniform, 60-Hz Electric and Magnetic Fields

A radiofrequency technique was used to determine the induced-current densities in humans and swine exposed to 60-Hz electric and magnetic fields.

By the end of the first year of this project, we constructed a large (3.66 x 3.66 x 2.44-m) cavity, operating at a resonant frequency of 57.3 MHz in the TE_{101} mode. Phantom tissues of different conductivities were characterized, using a slotted line. Prolate spheroids and spheres, as well as scaled human models composed of phantom tissue, were exposed in the region of maximum electric field strength in the center of cavity, and induced-current densities were compared. Semi-prolate spheroids and scaled human models were also exposed when in contact with the ground plane. The experiments demonstrated that cavity exposure can simulate both free space and ground-plane exposure conditions.

During the past 9 months, we have completed our dosimetry experiments. Three scaled human models (scale factors: 3.95, 5.95 and 8.25), composed of phantom tissue with conductivities of 1 and 10 S/m, were exposed both in the center of the cavity and with their feet in electrical contact with the ground plane. Thermograms were made using both a standard and a narrow-angle lens to obtain detailed temperature-rise data. PDP 11/34 computer software was developed to analyze the thermograms and provide automatic plots and printouts of the processed data. The induced-current densities were evaluated in terms of the measured temperature data. Figures 4A and 4B show a representative, computer-processed, induced-current pattern in a human model exposed to a 1-kV/m, 60-Hz electric field while standing on a ground plane. It should be noted that, in Figure 4, the data are presented in terms of induced-current density rather than temperature pattern, as in previous reports. The two-dimensional computer plots

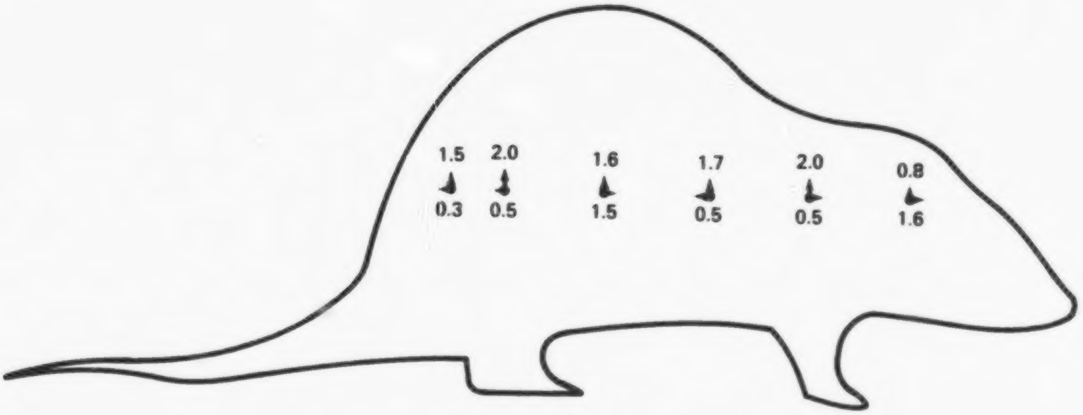


FIGURE 2. Measured Current Densities (nA/cm^2) in the Sagittal Plane of a Conducting Rat Model Exposed to a 1-kV/m, 60-Hz Electric Field. All four feet of the model were grounded, with equal currents passing through them.

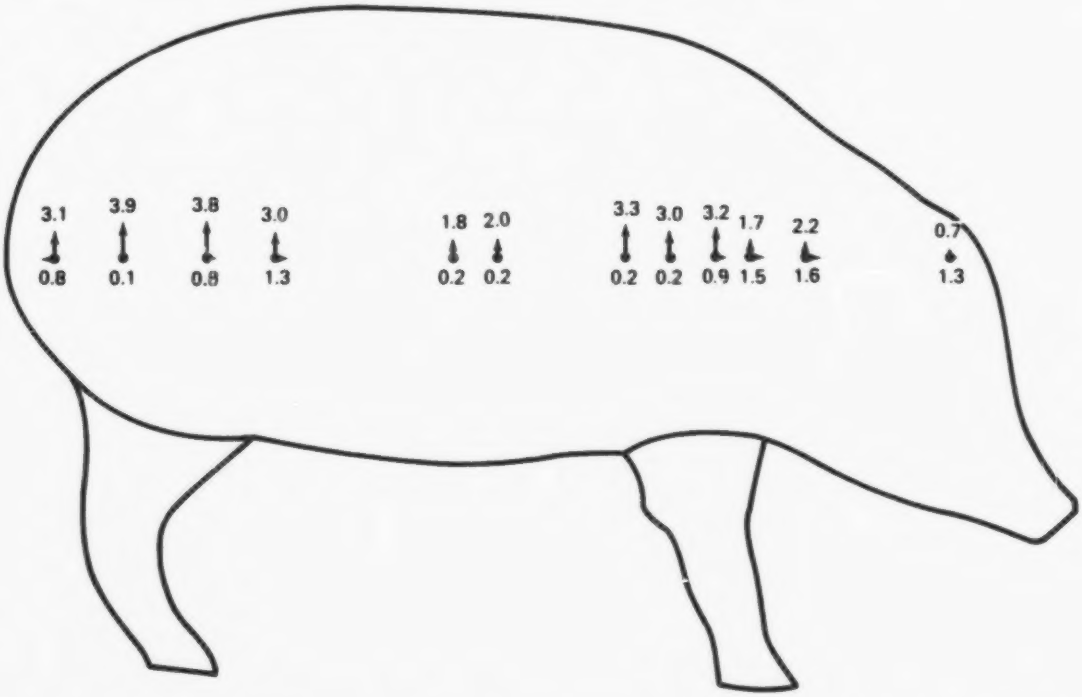
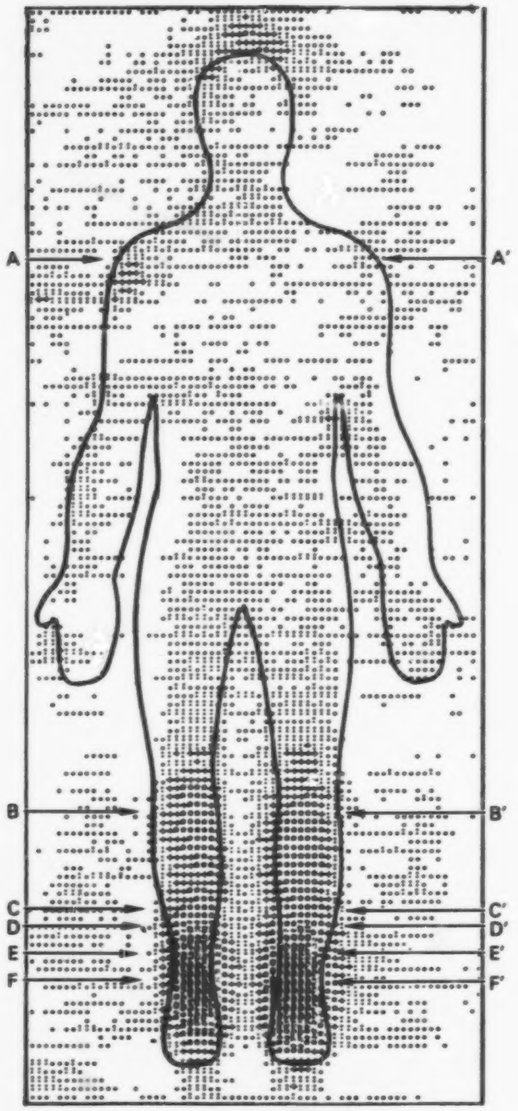


FIGURE 3. Measured Current Densities (nA/cm^2) in the Sagittal Plane of a Conducting Swine Model Exposed to a 1-kV/m, 60-Hz Electric Field. All four feet of the model were grounded, with equal currents passing through them.

provide considerably more quantitative data than provided by the previous photographs. The maximum induced-current densities within the body were located in the neck, knee, and ankle of each man. The induced-current density was independent of model size, a result predicted by long-wavelength analysis (the analysis used to extrapolate 57-MHz data to 60-Hz data). The induced-current densities in the 10-S/m models were in good agreement with the data reported by others. How-



= 0.000-25.813
• = 25.814-51.626
: = 51.627-77.440
+ = 77.441-103.254
* = 103.255-129.067
% = 129.068-154.881
@ = 154.882-180.695
@ = 180.696-206.508

FIGURE 4A. Pattern of Induced-Current Density in a 3.95-Scale Human Model (1.74 m, 70 kg, σ = 10 S/m) Exposed on the Ground Plane to a 1-kV/m, 60-Hz Electric Field.

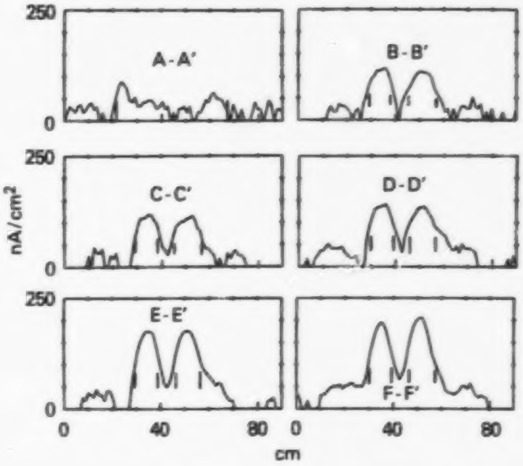


FIGURE 4B. B-Scans of Induced-Current Densities in Various Parts of the Human Model Described in Figure 4A.

ever, the current density in the legs of the 1-S/m models standing on the ground plane was unexpectedly low. This result was because the technique became invalid due to high fields in the legs of the model.

For free-space exposures, induced-current densities were the same in both the 1- and 10-S/m models. The induced-current densities at 60 Hz were tabulated for various regions of the human body (e.g., head, neck, heart, liver, kidney, pelvis, thigh, knee and ankle).

We also measured current distributions in swine models, with a conductivity of 1 S/m, which were exposed with their feet in electrical contact with the ground plane. In order to increase the heating in the torso of the model, a temperature-insensitive material was developed for the legs. The induced-current densities in various parts of the swine were also tabulated.

We have conducted a preliminary study of the current distributions in homogeneous human and swine models exposed to a magnetic field. The models filled with 1-S/m phantom material were exposed with feet in contact with the side wall of the cavity, where the maximum magnetic field vector was perpendicular to the frontal plane of the man and the sagittal plane of the swine. Heating patterns in man and swine models were obtained. The current distributions appeared as a circulating current passing through the legs of the models and through a ground-plane return path. High current densities were noted in the arms and the ankles of the human model and the back and legs of the pig model.

• In Vivo Mutagenesis of Electric Fields

Principal Investigator: B. J. McClanahan

Technical Assistance: L. I. Benton, R. L. Music, and S. A. Watson

Exposure to a 60-Hz electric field, at an unperturbed field strength of 100 kV/m, for 8 hr/day for 5 days, did not induce unscheduled DNA synthesis in mouse spermatocytes or impair the cells' ability to repair DNA damage induced by the mutagen, ethyl methanesulfonate. There was no detectable increase in abnormal sperm or numbers of sperm recovered from the caudae epididymides of exposed mice compared with those of the sham-exposed animals.

This project is concerned with the mutagenic potential of ac electric fields, such as those which might be associated with the transmission of electric power. We are investigating indications of mutagenicity or primary DNA damage by electric-field exposure in rats and mice. Electric-field-induced alteration of response to mutagens was evaluated by measuring the subsequent susceptibility of selected cell populations from exposed animals to chemical mutagens. This project complements the study, sponsored by the DOE Electric Energy Systems (EES) Division, on the effects of electric field exposure on rats and mice.

Unscheduled DNA Synthesis in Spermatocytes from Mice Exposed to A 60-Hz Electric Field

Unscheduled DNA synthesis (UDS) is the nonreplicative incorporation of ^{3}H-thymidine (^{3}H-TdR) into DNA. Potentially mutagenic lesions which require repair of the affected area of the genome may be detected by measuring the unscheduled incorporation of ^{3}H-TdR at times other than the stage of the cell cycle at which DNA synthesis normally occurs. The last scheduled DNA synthesis in male mouse germ cells occurs in preleptotene S spermatocytes just before meiosis. The spermatocytes then develop through a series of germ-cell stages for 28 to 30 days, until they leave the testes as late spermatids and enter the caput epididymis (or capita epididymides). The developing sperm reach the caudal epididymides 2-3 days later and enter the vas deferens after 2-3 days. If DNA from any of the meiotic or post-meiotic germ-cell stages is damaged, and UDS is induced, it can be detected by the incorporation of ^{3}H-TdR into the affected cells after they have matured into sperm appearing in the epidiymides and vas deferens.

In this study, 110 male Swiss-Webster mice were exposed for 8 hr (from 2300 to 0700 hr) for 5 days to a uniform, vertical, 60-Hz electric field at an unperturbed field strength of 100 kV/m. After the last exposure, the animals were injected intratesticularly with 50 μCi of ^{3}H-TdR (specific activity, 71 Ci/mmole). In addition, half of the exposed and half of the sham-exposed were injected intraperitoneally with 250 mg/kg body weight of ethyl methanesulfonate (EMS), a mutagen that induces UDS in mouse spermatocytes. Five animals from each group were killed at intervals over the next 14 days, and samples of purified sperm heads from the capita and caudae epididymides and vas deferens were assayed for their ^{3}H-TdR content.

No differences were detected in the incorporation of ^{3}H-TdR by spermatocytes in the sham-exposed and exposed animals (Figure 1), indicating that exposure to the electric field did not cause detectable DNA damage. The level of UDS evoked by administration of EMS did not differ between the two groups of animals. The ^{3}H-TdR content of the sperm heads was approximately 8 times higher in both groups of animals that received EMS. Preleptotene S cells that were synthesizing DNA at the time of ^{3}H-TdR administration are represented by the sharp peaks occurring on days 29-30, 34 and 37 in capita and caudae epididymides and vas deferens, respectively. The apparent increase in peak concentration of ^{3}H-TdR per million sperm heads in caput and caudal sperm from the exposed animals was not statistically significant. There appeared to be a slight reduction in the average ^{3}H-TdR content per sperm from the EMS-treated animals. This may have been due to a transient reduction in the rate of spermatocyte development, resulting in proportionately fewer labeled sperm in the EMS-treated animals.

The results of this study, which examined UDS during the first 6 hr after cessation of exposure, suggest that electric field

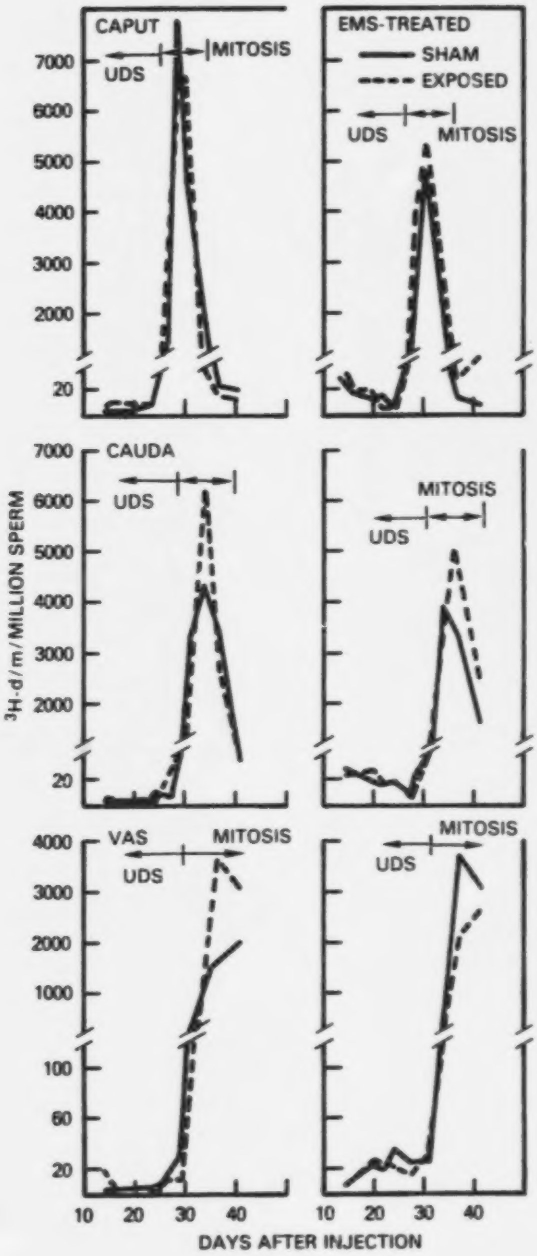


FIGURE 1. Unscheduled DNA Syntheses (UDS) in Mouse Germ Cells in the Reproductive Tract. Half of exposed and sham-exposed animals received the mutagen ethyl methanesulfonate (EMS).

exposure neither induced UDS in mouse spermatocytes, impaired the cells' ability to repair DNA damaged by EMS, nor increased the EMS-induced DNA lesions.

Abnormal Sperm and Numbers of Sperm from Mice Exposed to a 60-Hz Electric Field

One hundred twenty Swiss-Webster mice were exposed for 8 hr/day (2300 to 0700 hr) for 5 days to a vertical, 60-Hz, electric field at an unperturbed field strength of 100 kV/m. Immediately after the last exposure, half of the exposed animals and half of an equal number of sham-exposed animals were injected intraperitoneally with 250 mg/kg body weight of EMS. The animals were killed at intervals over the next 64 days, and sperm from the caudae epididymides were counted, then examined for abnormalities.

There were no remarkable differences in the numbers of sperm recovered from the exposed and sham-exposed animals at any time (Figure 2). A dramatic reduction in sperm numbers on day 31 after exposure was observed in both groups of animals that received EMS; by day 38 the numbers of sperm had returned to baseline levels.

Measurements of sperm abnormalities are in progress. Smears of sperm have been prepared weekly throughout the 9-wk experiment described above. The results obtained, at 9 and 38 days after the last day of exposure, on 500 cells from each of five mice per group did not reveal any obvious differences between the exposed and sham-exposed animals (Table 1). On day 9 there was a small, statistically significant ($P < 0.05$) increase in the numbers of abnormal sperm in the exposed animals. The EMS-treated animals had more abnormal sperm on both days 9 and 38, but no remarkable differences appeared between sperm of the exposed and sham-exposed groups.

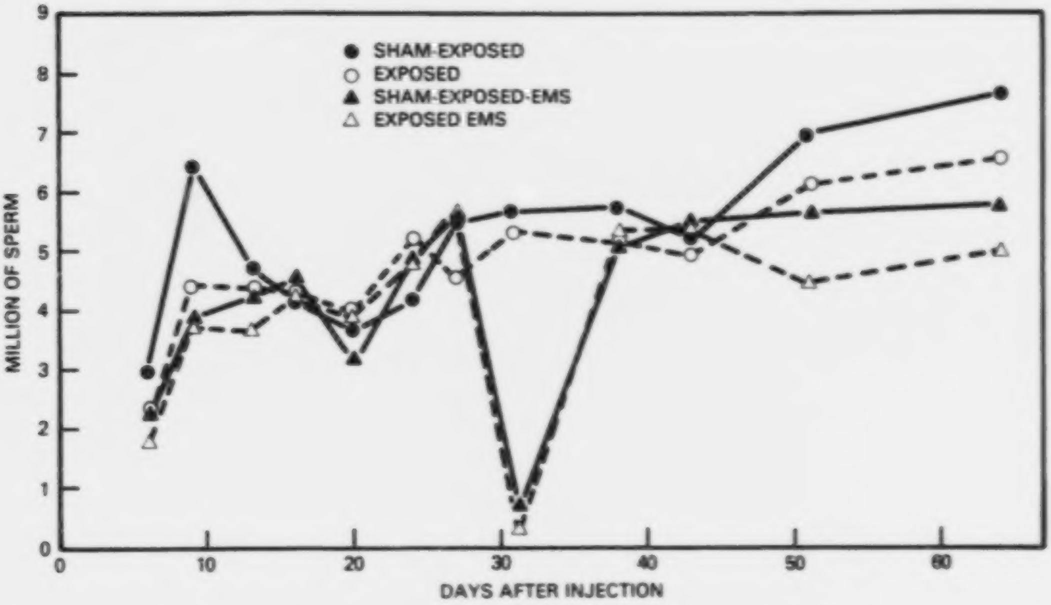


FIGURE 2. Number of Sperm Recovered from Caudae Epididymides of Mice Exposed to a 60-Hz Electric Field. Half of exposed and sham-exposed animals received the mutagen ethyl methanesulfonate (EMS).

TABLE 1. Sperm Abnormalities in Mice Exposed to a 60-Hz Electric Field. Half of exposed and sham-exposed animals received the mutagen ethyl methanesulfonate (EMS).

			EMS-Treated	
Day	Exposed	Sham-Exposed	Exposed	Sham-Exposed
9	5.2 ± 0.6	2.7 ± 0.2[a]	4.8 ± 0.9	5.4 ± 1.1
38	2.1 ± 0.3	3.2 ± 0.2	5.0 ± 0.3	8.0 ± 0.6

[a] Mean percent ± SE

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• In Vitro Effects of Electric Fields

Principal Investigator: M. E. Frazier

Other Investigator: J. E. Samuel

This project seeks to measure cytotoxicity, DNA damage and/or specific gene mutation resulting from exposure of mammalian cells in suspension to 60-Hz electric fields. Exposure of CHO-K1 cells to 60-Hz electric fields as high as 3.1 V/m for 48 hr did not increase the observable mutation frequency at a specific gene locus (HGPRT). However, exposure of CHO-K1 cells to 3.1 V/m for 1 hr resulted in a significant decrease in cell survival as measured using a clonal growth assay. Furthermore, electric-field exposure of this same cell line to 0.6 V/m for 24 hr also resulted in a significant reduction of cell survival. Preliminary studies indicate that exposure to a 0.1-V/m, 60-Hz electric field for 24 hr did not significantly affect cell survival as measured in our assay system. We believe that, in order to establish a dose-response relationship, it will be necessary to expose the cells to electric fields in excess of 10 V/m. To achieve this goal we have designed and are currently testing a new type of cell exposure system using carbon electrodes, with an agar bridge between the electrodes and the cell culture chamber.

In order to measure possible biological effects created by high-voltage transmission lines, we have developed a new method for exposing cells, in vitro, to 60-Hz electric fields. The exposure system (Figure 1) is a simple transformer, with a 60-Hz voltage source connected to the primary winding, and a cell suspension in a toroidal containment vessel forming the secondary winding. The siliconized glass containment vessel has an agar-medium base layer and a cell-culture-medium top layer; both layers have the same conductance. We have used this system to generate data on the effects of 60-Hz electric fields (up to 3.1 V/m) on cell viability, growth rate, and mutation frequency using Chinese hamster ovary (CHO-K1) as the indicator cell line (Figure 2).

Initial survival was determined using CHO cells in a clonal growth assay. This assay requires the seeding of a known number of monodispersed cells (at low cell density) from each exposure group into suitable culture dishes containing growth medium, and counting the resultant clones. The mutation assay also uses CHO cells to score specific mutations at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus.

This year's data (Table 1) confirm last year's preliminary results. First, the cloning efficiency of CHO-K1 cells was significantly reduced following exposure to 3.1 V/m, indicating increased cytotoxicity due to electric-field exposure. Second, the mutation frequency of CHO-K1 cells exposed to a 3.1-V/m electric field was not significantly different from that of unexposed or sham-exposed cells.

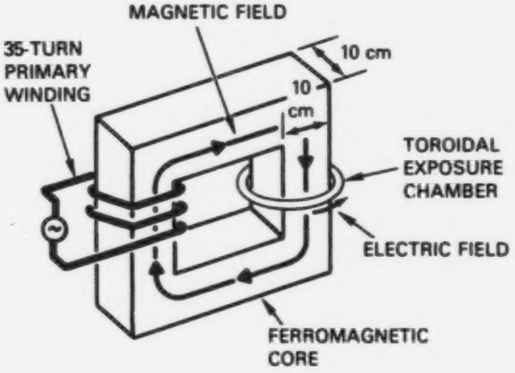

FIGURE 1. System for Exposing Cell Cultures to Electric Fields. Current applied through the 35-turn primary winding produces a magnetic field in the ferromagnetic core. An electric field is generated by the 60-Hz magnetic field (Faraday's law of induction). Cells in the toroidal chambers are exposed to the 60-Hz electric field along with a contaminating magnetic field (≤ 0.1 gauss). Control for potential magnetic-field effects is achieved by incorporating a second toroidal chamber, insulated from the electric field.

In an attempt to show a dose-effect relationship or threshold effect, we carried out a series of experiments to determine if the duration of electric-field exposure was an important factor. As can be seen from Table 2, exposure to a 3.1-V/m electric field for either 1 or 24 hr significantly decreased survival of exposed cells in comparison to exposed control cells.

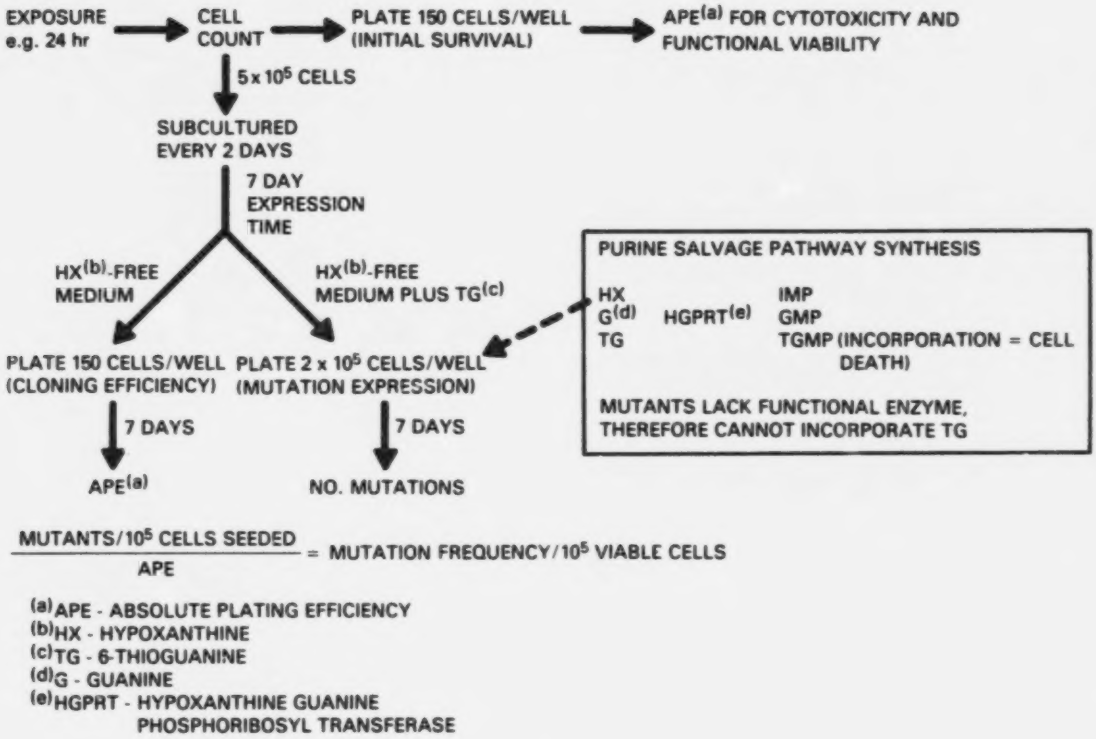
EXPOSURE e.g. 24 hr
CELL COUNT
PLATE 150 CELLS/WELL (INITIAL SURVIVAL)
APE(a) FOR CYTOTOXICITY AND FUNCTIONAL VIABILITY
5 x 10^5 CELLS
SUBCULTURED EVERY 2 DAYS
7 DAY EXPRESSION TIME
HX(b)-FREE MEDIUM
HX(b)-FREE MEDIUM PLUS TG(c)
PLATE 150 CELLS/WELL (CLONING EFFICIENCY)
PLATE 2 x 10^5 CELLS/WELL (MUTATION EXPRESSION)
7 DAYS
7 DAYS
APE(a)
NO. MUTATIONS
PURINE SALVAGE PATHWAY SYNTHESIS
HX
G(d)
TG
HGPRT(e)
IMP
GMP
TGMP (INCORPORATION = CELL DEATH)
MUTANTS LACK FUNCTIONAL ENZYME, THEREFORE CANNOT INCORPORATE TG
MUTANTS/10^5 CELLS SEEDED / APE = MUTATION FREQUENCY/10^5 VIABLE CELLS
(a)APE - ABSOLUTE PLATING EFFICIENCY
(b)HX - HYPOXANTHINE
(c)TG - 6-THIOGUANINE
(d)G - GUANINE
(e)HGPRT - HYPOXANTHINE GUANINE PHOSPHORIBOSYL TRANSFERASE

FIGURE 2. Procedure for Determining Plating Efficiency and Mutation Frequency of CHO-K1 Cells Exposed to Electric Fields.

TABLE 1. Effects of 60-Hz ac Electric Fields (3.1 V/m) on Plating Efficiency and Mutation Frequency of CHO-K1 Cells.

		Plating Efficiency, ± SE[a]				Mutation Frequency/ 10^5 Viable cells			
Experiment No.	Exposure Time, hr	Exposed	Sham-Exposed	Control[b]	p[c]	Exposed	Sham-Exposed	Control[b]	p[c]
81-1	24	43.1 ± 1.2	52.2 ± 1.4	54.1 ± 2.3	<0.001	7.8	12.8	6.2	NS[d]
81-2	48	58.4 ± 2.1	72.0 ± 1.8	69.9 ± 1.0	<0.001	--	--	--	--
81-3	24	61.7 ± 1.1	73.4 ± 1.3	73.4 ± 2.5	<0.001	0.21	0.30	0.23	NS
81-4	24	74.2 ± 2.5	98.0 ± 2.0	101.9 ± 3.4	<0.001	0.38	0.12	0.32	NS
81-5	48	69.2 ± 2.1	73.8 ± 2.3	71.9 ± 2.3	<0.05	1.03	1.22	1.13	NS

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[a] Number of clones formed/150 cells seeded; 72 plates were used in making each determination. The numbers are ± standard error.

[b] Culture grown on agar in incubation

[c] Level of significance between exposed and sham-exposed, using a 2-tailed, unpaired *t*-test. Values obtained from published tables of the distribution of *t*.

[d] Not significant at the 0.05 level

TABLE 2. Effects of 60-Hz ac Electric Fields (3.1 V/m) on Plating Efficiency of CHO-K1 Cells as a Function of Exposure Time.

Exposure Duration, hr	Absolute Plating Efficiency, ± SE[(a)]		p[(b)]
	Exposed	Sham-Exposed	
0	50.0 ± 1.7	48.0 ± 1.4	NS[(c)]
1	59.4 ± 1.9	63.7 ± 1.4	<0.05
8	59.9 ± 1.4	75.8 ± 1.5	<0.001
16	53.4 ± 1.2	58.3 ± 1.1	<0.001
24	(Several Experiments)		<0.001
48	(2 Experiments)		<0.05 <0.001

[(a)] Number of clones formed/150 cells seeded; 72 plates were used in making each determination.

[(b)] Level of significance between exposed and sham-exposed, using a 2-tailed unpaired *t*-test. Values obtained from published tables of the distribution of *t*.

[(c)] NS = not significant at 0.05 level.

In our attempt to establish a threshold based on electric-field strength, our preliminary studies indicate a significant effect on cell survival, for 24 hr, at 0.6 V/m, but not at 0.1 V/m.

Another aspect of our studies this year has been to develop and test a new exposure system. The present exposure system offered several advantages over the previous method, which used platinum electrodes directly coupled to the culture medium. Unfortunately, we are approaching the upper limits of field strength for the new, indirectly coupled exposure system. To date, the only effect apparent is a decrease in survival of cells following their removal from the field and subsequent plating in media at low cell densities. As a result of these findings, and because of the limitations of the present system, we are designing and testing still another exposure system which will allow us to test higher-strength electric fields. This system employs carbon electrodes directly coupled to the culture medium. In order to assure that electrode effects do not perturb the experiment, we have interposed agar bridges between the electrodes and the cell suspension. These agar bridges contain ionic constituents identical to those in the cell-culture medium. Both the bridges and the chemical nature of the electrodes (carbon instead of platinum) help to eliminate the problems of ion migration and cytotoxicity observed when platinum electrodes were coupled directly to the medium.

Preliminary experiments with the new carbon-electrode exposure chamber have shown the system to have several advantages: 1) The cost and ease of assembly of these chambers allow the use of more exposure chambers per experiment. 2) The agar-medium interface in the carbon-electrode chamber allows for longer cell exposures (up to 72 hr) under optimal growth conditions. Furthermore, the design of the exposure system facilitates sampling, improving our ability to conduct more accurate growth-rate (and other) studies requiring multiple sampling. 3) Most importantly, we anticipate that this exposure method will allow us to obtain field strengths 10 to 15 times greater than those now obtainable with the magnetic induction exposure system. This will allow both confirmation and extension of our understanding of the biological effect we observed with magnetically induced electric fields. 4) Finally, this versatile system can be used with ac or dc power supplies, and for studying biological effects of pulsed electric fields.

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Biomedical studies in the fission energy technology area are, for the most part, directed toward evaluation of long-term effects from internal exposure to radionuclides. These include studies in progress for many years in dogs that have inhaled various plutonium compounds and a newly instituted study involving inhalation of americium oxide. The inhalation toxicity of a wider variety of actinide compounds is being studied in rodents, including an attempt to evaluate the carcinogenicity of inhaled plutonium at life-span radiation doses to the lung of 2 to 10 rad. Both dogs and rodents are employed in continuing studies of the effects of chronic exposure to simulated uranium mine atmospheres.

Studies in rats and dogs have shown that cigarette smoke enhances retention of inhaled plutonium in the lung, and are being extended to evaluate effects on carcinogenesis. The toxicity of sodium or lithium that might be accidently released, from a liquid metal fast breeder reactor or from a fusion reactor, and the toxicity of mixed aerosols of plutonium and sodium are under investigation. A study of the toxicity of chronically inhaled krypton-85 which may be released in small amounts from nuclear reactors, is being concluded. The biological behavior and carcinogenicity of high-specific activity uranium and protactinium isotopes that might be released from reactors employing a thorium fuel cycle are being investigated.

Studies related to the exposure of the general public include those concerned with the effects of age on the toxicity of incorporated radionuclides, and the effect of age, and other factors, on the gastrointestinal absorption of radionuclides. In the medical area, a device developed to irradiate the blood, which may be a useful treatment for leukemia or for the prevention of transplant rejection, will soon be ready for clinical trials.

In all of these areas, the development of new experimental techniques plays an important role. This has included major efforts in aerosol generation and exposure technology, and increasing emphasis on the computer handling and statistical analysis of animal toxicology data.

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Aerosol Technology Development

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Technical Assistance: B. R. Garrity and J. R. Laidler

A variety of tasks are included in this project, all designed to improve the capabilities of this laboratory to conduct animal inhalation exposures to airborne pollutants, and to characterize the aerosols used. These tasks include development of an improved, nose-only aerosol exposure chamber for rats; improvement of a new dry-dust feeder for aerosol generation; measurement of the surface area of volcanic-ash particles; development of a new way to describe aerosol particle-size distribution which can be used with programmable pocket calculators; and comparative testing of a method for inhalation exposure chamber evaluation.

Nose-Only Aerosol Exposure Chamber

We have demonstrated that a newly developed, nose-only exposure chamber for rats provides a more uniform exposure than the standard chamber in use at PNL, resulting in less variability in the amount of aerosol deposited per rat. Much of the remaining variability is due to individual differences in the animals.

In standard, nose-only, rat exposure chambers the animals exhale into the chamber; this air, depleted in aerosol, is passed on to animals downstream, resulting in a reduced aerosol dose to those animals. In our new design, fresh aerosol flows past each rat's nose and is then exhausted from the chamber (Figures 1A and 1B), exposing all animals to the same concentration of aerosol.

In a recent experiment, three groups of rats were exposed to ^{169}Yb-tagged $^{239}PuO_2$ aerosol in each type of chamber. (The ^{169}Yb, a gamma emitter, can be easily detected in a living animal.) In vivo counting was used to measure the deposited activity. Figures 2A and 2B show histograms derived from measured radionuclide activity in 105 rats exposed in the standard chambers and 55 rats exposed in the new chambers.

In many inhalation toxicology experiments, a problem arises because deposited aerosols cannot be easily detected in live animals, therefore the deposited dose cannot be determined until the animals are sacrificed. Because of the variability in deposited dose, the investigator must expose (and maintain throughout the study) "extra" animals to insure sufficient numbers in each dose group. The new chamber design will reduce dose variability (Table 1), thereby decreasing the number of "extra" animals required and associated costs.

Redesign of Dry-Dust Feed Mechanism

A dual-brush dust-feed mechanism consists of a flexible-bristle, random-wound brush (hopper brush) to keep the dust in the hopper from settling, and a flexible-bristle, spirally wound brush (feed brush) to transport and meter dust from the hopper. During long-term generation of organic powders, the output rate became unpredictable due to electric charge (Figure 3A). When all metal parts of the dust-feed mechanism (including the central wires in the nylon hopper and feed brushes) were grounded, the unit operated as expected (Figure 3B). The latest model is machined from a single aluminum block and does not consist of a separate hopper and feed tubes. Since all parts are electrically connected, grounding the block grounds the entire mechanism.

Determination of Particle Size Distribution Using a Programmable Pocket Calculator

Aerosol sample masses often follow normal distributions, while aerosol particle sizes often follow log-normal distributions. Investigators usually report the estimated mean and standard deviation of sample mass or the estimated median and geometric standard deviation of particle diameter. Errors associated with these estimates are useful, but are not usually reported because error calculation requires a computer with an extensive memory. The logistic distribution is a close approximation to the normal distribution, and its inverse probability distribution function has a closed form (the logit

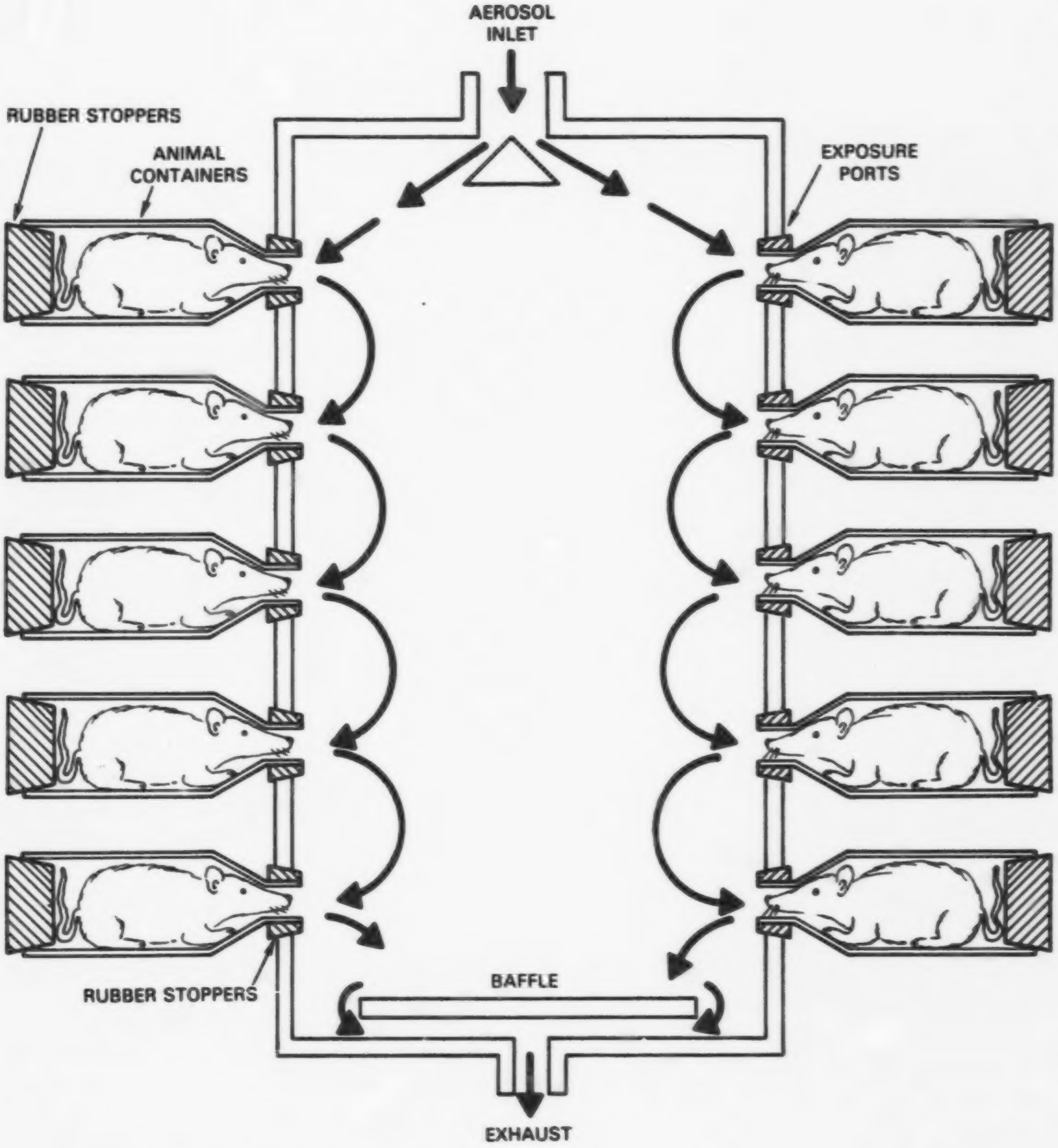
AEROSOL INLET
RUBBER STOPPERS
ANIMAL CONTAINERS
EXPOSURE PORTS
RUBBER STOPPERS
BAFFLE
EXHAUST

FIGURE 1A. Standard Rat Exposure Chamber.

transformation), which can be calculated on programmable pocket calculators with modest memories. Therefore, using the logistic or log-logistic distribution, one can estimate the probable errors in parameters more easily and with simpler equipment.

As an example, we have described the particle size distribution of a NaCl aerosol (Table 2), first assuming a log-normal distribution, then a log-logistic distribution with a coordinate transformation (16X/15) to approximate the log-normal distribution. The results are contrasted in Table 3. The log-normal distribution function gives a smaller confidence region; however, the estimates from the log-logistic approximation are within the log-normal confidence region. The converse is also true.

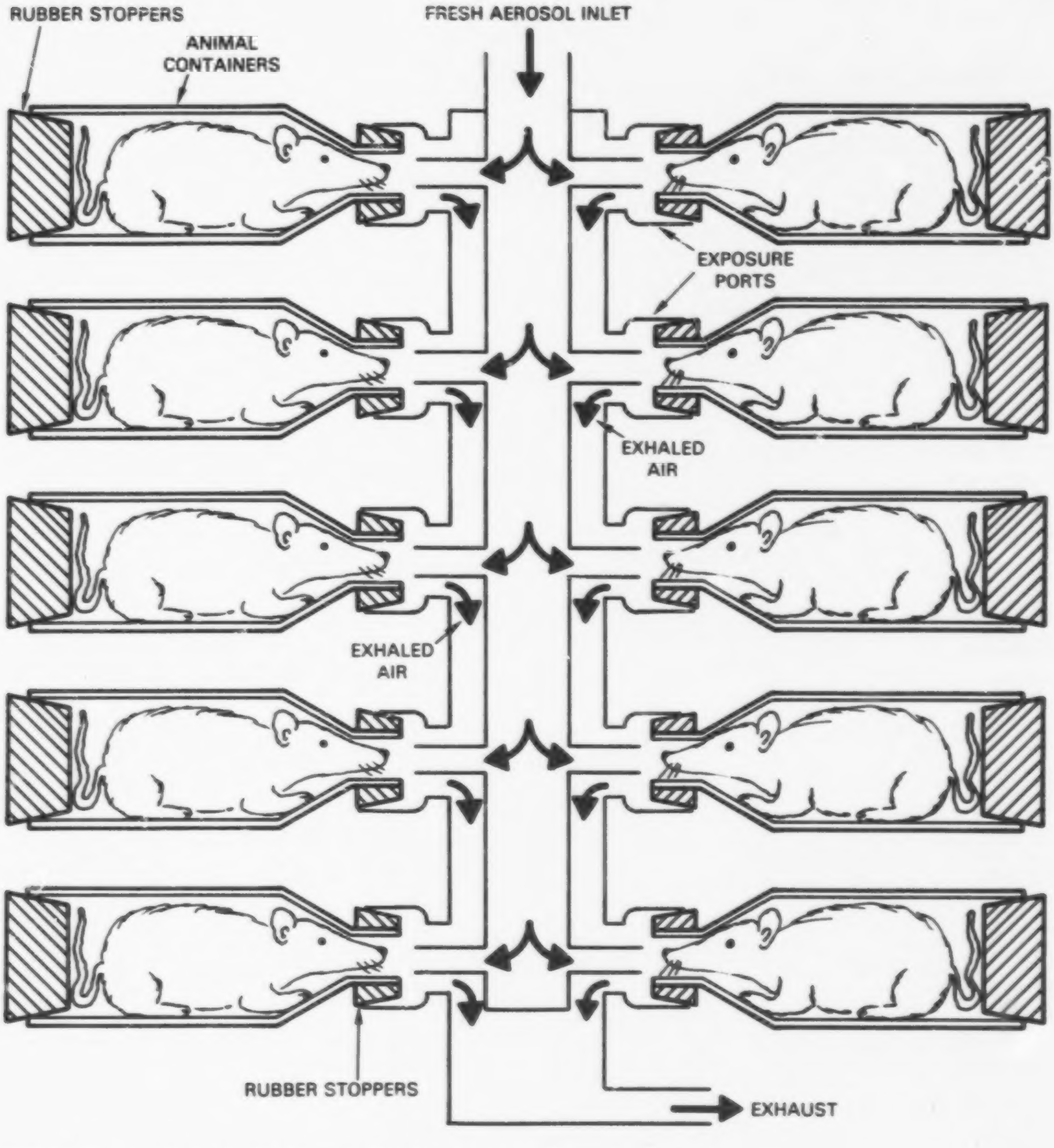
RUBBER STOPPERS
FRESH AEROSOL INLET
ANIMAL CONTAINERS
EXPOSURE PORTS
EXHALED AIR
EXHALED AIR
RUBBER STOPPERS
EXHAUST

FIGURE 1B. New Design for Rat Exposure Chamber that Avoids Rebreathing Aerosol.

Test for Evaluating Performance of Aerosol Exposure Chambers

We have cooperated with investigators at Inhalation Toxicology Research Institute and the University of Vermont to develop a simple test to evaluate performance of inhalation toxicology exposure chambers. Three approaches were compared for evaluating chamber performance with respect to effects of animal loading, chamber flow rate and incoming air temperature on uniformity of aerosol concentration. These approaches were modeling, full-scale point tests and full-scale dynamic tests. In modeling experiments, water flows through scale models of chambers, and we use colored-dye injections to observe flow patterns and mixing characteristics of the chambers. Full-scale point tests involve simultaneous collections of aerosol samples at a sufficient number of locations

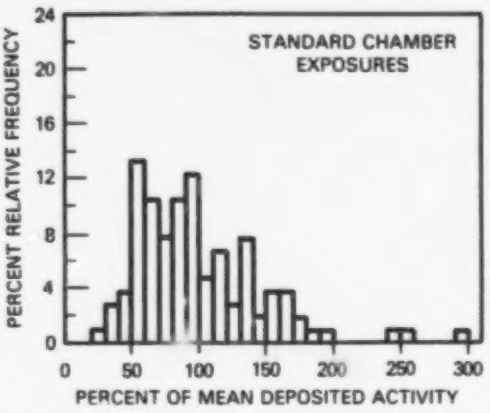

FIGURE 2A. Standard Exposure Chamber Deposition Data.

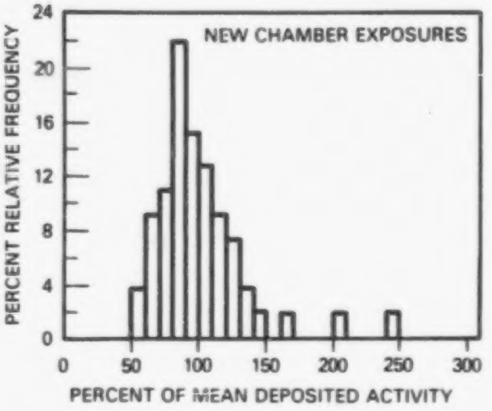

FIGURE 2B. Newly Designed Exposure Chamber Deposition Data.

TABLE 1. Reduction of Dose Variability Among Animals by New Design for Inhalation Exposure Chambers.

	Yield/36 Animals in Range Given, %				
Chamber	± 50	± 40	± 30	± 20	± 10
Standard	27	22	16	12	6
New Design	33	32	28	20	10

to establish uniformity of concentration in the chamber. Full-scale dynamics tests involve measuring concentration versus time near the inlet and outlet of a chamber when a bolus of tracer material passes through it. Analysis of these data yields percent dead-space and the coefficient of dispersion of the chamber. Both these quantities are indicators of how uniform an exposure atmosphere will be. Comparison of results from the various laboratories is in progress.

Measurement of Particle Surface Area

We have recently developed the capability to measure specific surface area and density of powdered materials. The Brunauer, Emmet and Teller (BET) gas adsorption technique uses a microbalance to measure the mass of nitrogen adsorbed onto the surface of the sample at a given nitrogen

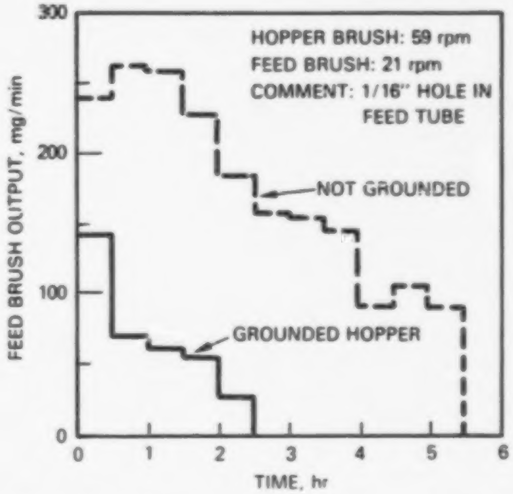

FIGURE 3A. Comparison of Output Between Grounded and Ungrounded Dry Dust-Feed Mechanism.

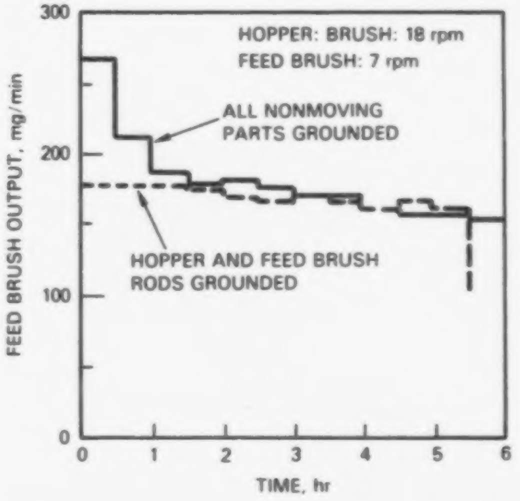

FIGURE 3B. Effect of Grounding Moving and Nonmoving Parts of Feed Brush on Output Stability.

TABLE 2. Cascade Impactor Data for NaCl Aerosol.

Cutoff Diameter, µm	Cumulative Percent Less Than D (By Mass)
0.29	0.5
0.51	3.6
0.79	12.4
1.70	36.4
2.67	58.4
3.92	85.7
5.90	94.1
8.11	97.1

TABLE 3. Comparison of Estimated Particle Size Distribution by Two Methods (95% Confidence Limits).

	Log Logistic (Simple Approximation to Log-Normal)			Log-Normal		
Parameter	Estimate	Min	Max	Estimate	Min	Max
MMAD	2.03	1.87	2.20	1.97	1.85	2.11
GSD	2.04	1.94	2.16	2.10	2.01	2.20
R^2	0.991	--	--	0.995	--	--

pressure. The relationship between pressure and the mass of nitrogen per unit area determines the surface area of the sample. Presently, at least 10-mg samples are needed for high specific-surface-area powders, and even larger samples are required for low specific-surface-area materials. Smaller samples would be more convenient to use, expecially for radioactive and/or highly toxic materials. We therefore plan to investigate ways of reducing sample size.

After calibrating our equipment with standard powders of known specific surface area, we characterized samples of Mount St. Helens volcanic ash (Table 4). The high specific-surface-area values are probably due to the roughness of the particle surface and the numerous very small particles adhering to those surfaces.

TABLE 4. Specific Surface Area and Density of Mount St. Helens Ash.

Ash Collection Site	Size Fraction	Specific Surface Area, m^2/g	Density, g/cm	Mass of Sample, mg
Composite[a]	Respirable	2.95 ± 0.11	3.76-0.16	50.4
Yakima, WA	Respirable	13.48 ± 0.18	--	15.05
Yakima, WA	<20 µm	10.20 ± 0.12	3.33	30.07
Yakima, WA	Coarse: >3.5 µm	1.46 ± 0.02	2.75	310.07
Missoula, MT	Respirable	8.69 ± 0.25	3.43	23.54
Missoula, MT	>20 µm	1.53 ± 0.09	2.56	187.71

[a] Mixed samples from Yakima, Ritzville and Spokane, WA

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• Inhaled Plutonium Oxide in Dogs

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This project is concerned with long-term experiments to determine the lifespan dose-effect relationships of inhaled $^{239}PuO_2$ and $^{238}PuO_2$ in beagles. The data will be used to estimate the health effects of inhaled transuranics.

Beagle dogs given a single exposure to $^{239}PuO_2$ or $^{238}PuO_2$ aerosols are being observed for lifespan dose-effect relationships. The ^{239}Pu body burden of the nine dogs that died of pulmonary-fibrosis-induced respiratory insufficiency during the first 3 yr after exposure was 1 to 12 μCi; one of these dogs had a pulmonary tumor. Twenty-seven additional dogs, with body burdens ranging from 0.05 to 1.8 μCi, died due to pulmonary neoplasia 3 to 10 yr after exposure. Nineteen of the dogs exposed to ^{238}Pu have died during the first 7½ yr after exposure due to bone and/or lung tumors; their body burdens at death ranged from 0.7 to 10 μCi. Chronic lymphopenia was the earliest observed effect after inhalation of $^{239}PuO_2$ or $^{238}PuO_2$, occurring 0.5 to 2 yr after deposition of ≥80 nCi plutonium in the lungs.

To determine the lifespan dose-effect relationships of inhaled plutonium, 18-mo-old beagle dogs were exposed to aerosols of $^{239}PuO_2$ (mean AMAD, 2.3 μm; mean GSD, 1.9), prepared by calcining the oxalate at 750°C for 2 hr; or to $^{238}PuO_2$ (mean AMAD, 1.8 μm; mean GSD, 1.9), prepared by calcining the oxalate at 700°C and subjecting the product to $H_2{}^{16}O$ steam in argon exchange at 800°C for 96 hr. This material, referred to as pure plutonium oxide, is used as fuel in space-nuclear power systems.

One hundred thirty dogs exposed to $^{239}PuO_2$ in 1970 and 1971 were selected for long-term studies; 22 will be sacrificed to obtain plutonium distribution and pathology data, and 108 were assigned to lifespan dose-effect studies (Table 1). One hundred thirteen dogs exposed to $^{238}PuO_2$ in 1972 and 1973 were selected for lifespan dose-effect studies (Table 2). Twenty-four additional dogs were exposed for periodic sacrifice. The appendix (following the entire Annual Report) shows the status of the dogs on these experiments.

During the first 10 yr following exposure to $^{239}PuO_2$, all of the dogs in the highest-level dose group and in Dose Level Group 5, ten in Group 4, four in Group 3, two in Group 2 and four in Dose Level Group 1 were euthanized when death was imminent. Fourteen dogs were sacrificed for comparison of plutonium tissue distribution.

TABLE 1. Lifespan Dose-Effect Studies with Inhaled $^{239}PuO_2$ in Beagles(a).

Dose Level Group	Number of Dogs		Initial Alveolar Deposition(b)	
	Male	Female	nCi(c)	nCi/g Lung(c)
0	10	10	0	0
1	10	10	3.5 ± 1.3	0.029 ± 0.011
2	10	10	22 ± 4	0.18 ± 0.04
3	10	10	79 ± 14	0.66 ± 0.13
4	10	10	300 ± 62	2.4 ± 0.4
5	10	10	1100 ± 170	9.3 ± 1.4
6	3	5	5800 ± 3300	50 ± 22
	63	65		

(a)Exposed in 1970 and 1971

(b)Estimated from external thorax counts at 14 and 30 days postexposure and estimated lung weights (0.011 x body weight)

(c)Mean ± 95% confidence intervals around the means

Table 3 and Figure 1 show the primary causes of death and the distribution of ^{239}Pu in the tissues of these animals. One 80-mo-old control dog was euthanized because of an oral tumor.

TABLE 2. Lifespan Dose-Effect Studies with Inhaled $^{238}PuO_2$ in Beagles[a].

Dose Level Group	Number of Dogs		Initial Alveolar Deposition[b]	
	Male	Female	nCi[c]	nCi/g Lung[c]
0	10	10	0	0
1	10	10	2.3 ± 0.8	0.016 ± 0.007
2	10	10	18 ± 3	0.15 ± 0.03
3	10	10	77 ± 11	0.56 ± 0.07
4	10	10	350 ± 81	2.6 ± 0.5
5	10	10	1300 ± 270	10 ± 1.9
6	7	6	5200 ± 1400	43 ± 12
	67	66		

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[a] Exposed in 1973 and 1974

[b] Estimated from external thorax counts at 14 and 30 days postexposure and estimated lung weights (0.011 x body weight)

[c] Mean ± 95% confidence intervals around the means

As survival time increased, the fraction of plutonium in the lung decreased to ~15% of the final body burden by 9 to 10 yr after exposure. During the first postexposure year, plutonium was translocated primarily to the thoracic lymph nodes, with little plutonium translocated to other tissues. Plutonium content of the thoracic lymph nodes was ~50% of the final body burden at 9-10 yr after exposure; the abdominal lymph nodes, principally the hepatic nodes, contained ~11%. The fraction of plutonium in liver increased, accounting for ~22% of the final body burden at 9 and 10 yr after exposure in the higher (0.3- to 0.4-μCi body burden) dose-level groups. The organ distribution of plutonium in the periodically sacrificed dogs was generally similar to that of the high-dose-level dogs euthanized when death was imminent during the first 2 yr after exposure. The lower-dose-level dogs (0.001- to 0.2-μCi final body burden) sacrificed or euthanized during the 4th to 10th postexposure years had a much smaller fraction of the final body burden in the liver, with a larger fraction retained in the lungs and/or thoracic lymph nodes. About 2% of the final body burden was in the skeleton at 9 to 10 yr after exposure.

The dogs euthanized because of respiratory insufficiency during the 3-yr postexposure period had increased respiration rates, and hypercapnia and hypoxemia associated with lesions in the lungs. Intermittent anorexia and body weight loss accompanied the respiratory insufficiency. Histopathologic examination of the lungs showed radiation pneumonitis characterized by focal interstitial and subpleural fibrosis, increased numbers of alveolar macrophages, alveolar epithelial hyperplasia, and foci of squamous metaplasia. Autoradiographs showed activity primarily composed of large stars, more numerous in areas of interstitial and subpleural fibrosis. Dog 804M also had a pulmonary tumor, classified as a bronchiolar-alveolar carcinoma.

Twenty-eight of the 41 dogs euthanized 3 to 10 yr after exposure had lung tumors. Radiographic evidence of pulmonary neoplasia was observed before respiratory insufficiency developed. However, respiratory insufficiency was frequently observed prior to euthanasia due to neoplasia in the lung. All of the dogs with lung tumors were in Dose Level Groups 3, 4, 5, and 6. Dogs 794M, 803M, 824F, and 835F (Dose Level 4), 697M, 778M, and 834F (Dose Level 3), 754M and 769F (Dose Level 2), and 875M, 891M, 899F and 908M (Dose Level 1) died during the 7- to 10-yr postexposure period, of causes presently thought to be unrelated to plutonium exposure. Table 4 summarizes, by dose-level group, the mortality and lesions associated with death through 10 yr after exposure.

In 16 of the dogs, the lung tumors were classified as bronchiolar-alveolar carcinoma; in five dogs as adenosquamous carcinoma; in four dogs, adenocarcinoma; in one dog, epidermoid carcinoma; in one dog, epidermoid and bronchiolar-alveolar carcinoma; and in another dog, adenocarcinoma, adenosquamous carcinoma and bronchiolar-alveolar adenocarcinoma. The epidermoid carcinoma metastasized to the skeleton; the bronchiolar-alveolar carcinomas metastasized only to the thoracic lymph nodes in eight dogs, and to several organs (including thoracic lymph nodes, mediastinum, kidney, thyroid, skeleton, heart, adrenal gland, aorta, and axillary, prescapular, cervical, splenic and hepatic lymph nodes) in three other dogs. Two of the adenosquamous carcinomas metastasized to thoracic lymph nodes, mediastinum and thoracic pleura, and one to the hepatic and tracheobronchial lymph nodes. The adenocarcinoma was not metastatic. Three of the dogs had lesions of secondary hypertrophic osteoarthropathy. Sclerosing lymphadenopathy was associated with the high concentration of plutonium in the thoracic and hepatic lymph nodes. There was also a generalized lymphoid atrophy that may be related to debilitation in the dogs with respiratory insufficiency, or to lympho-

TABLE 3. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}PuO_2$.

Dog Number	Time After Exposure, Mo	Final Body Burden, μCi	Percent of Final Body Burden: Lungs	Thoracic Lymph Nodes[a]	Abdominal Lymph Nodes[b]	Liver	Skeleton	Cause of Death
478M	0.25	0.293	98	0.15	0.02	0.24	0.18	Sacrifice
435F	0.25	3.841	99	0.11	0.01	0.00	0.03	Scarifice
816M	0.50	0.399	99	0.12	0.01	0.00	0.03	Sacrifice
918M	1	0.074	99	0.82	0.02	0.11	0.08	Sacrifice
920F	1	0.011	94	0.47	0.03	0.08	0.61	Sacrifice
913M	1	4.849	98	1.1	0.00	0.03	0.05	Sacrifice
702F	5	1.682	94	5.7	0.00	0.01	0.09	Sacrifice
709M	5	1.726	97	2.2	0.00	0.00	0.05	Sacrifice
734M	5	0.914	96	3.4	0.00	0.01	0.05	Sacrifice
739F	5	1.511	95	4.7	0.03	0.00	0.00	Sacrifice
910M	11	12.229	84	15	0.01	0.06	0.05	Radiation Pneumonitis
747F	12	5.434	71	29	0.03	0.0[illegible]	0.07	Radiation Pneumonitis
906F	12	6.154	88	12	0.00	0.03	0.05	Radiation Pneumonitis
849F	13	0.0007	80	15	0.20	0.04	1.6	Sacrifice
896F	15	4.115	81	15	0.92	0.23	0.12	Radiation Pneumonitis
817M	21	3.794	64	34	0.13	1.4	0.19	Radiation Pneumonitis
815M	25	0.074	64	32	--	0.08	0.10	Sacrifice
829M	26	3.198	75	19	0.79	4.2	0.45	Radiation Pneumonitis
760M	31	0.978	71	23	0.57	3.7	0.28	Radiation Pneumonitis
890F	31	2.012	55	28	2.2	13	0.26	Radiation Pneumonitis
804M	37	1.101	62	29	0.19	7.9	0.36	Radiation Pneumonitis, Lung Tumor
798F	43	0.0056	55	44	0.02	0.17	0.43	Sacrifice
772M	53	1.821	42	22	0.88	29	0.69	Lung Tumor
759M	53	0.707	43	27	12	15	0.65	Lung Tumor
796F	55	0.671	40	31	4.1	21	1.0	Lung Tumor
783M	59	1.377	59	11	1.8	26	0.67	Lung Tumor
873M	62	1.746	45	27	6.4	16	0.76	Lung Tumor
753F	69	1.171	35	31	0.09	24	0.64	Lung Tumor
761M	69	1.064	36	37	6.3	19	0.53	Lung Tumor
727M	72	0.585	39	24	12	23	0.78	Lung Tumor
762M	72	0.0017	51	42	0.34	0.71	0.66	Sacrifice
837M	72	1.034	42	38	0.70	14	0.46	Lung Tumor
863F	76	0.617	33	12	1.3	47	1.4	Lung Tumor
852F	77	1.067	33	35	0.88	26	0.94	Lung Tumor
803M	79	0.415	20	46	11	20	1.4	Interstitial Pneumonitis
875M	83	0.0026	24	66	0.34	0.64	6.3	Malignant Lymphoma, Kidney
754M	84	0.0046	29	66	0.23	0.39	1.2	Status Epilepticus
835F	86	0.099	27	65	0.95	3.1	1.7	Reticulum Cell Sarcoma
880F	86	0.468	19	31	13	34	0.37	Lung Tumor
769F	90	0.019	36	57	0.32	1.7	1.8	Ovarium Tumor
888M	93	0.179	32	40	10	12	2.1	Lung Tumor
856F	94	0.306	40	45	0.78	9.0	3.9	Lung Tumor
889F	94	0.613	14	27	6.9	41	8.1	Lung Tumor
787M	95	0.473	24	19	12	39	2.7	Lung Tumor
820F	96	0.387	14	40	7.6	29	1.4	Lung Tumor
834F	97	0.025	30	46	17	3.5	0.91	Pyometra
752M	98	0.055	24	62	1.2	7.7	0.98	Lung Tumor
864F	100	0.616	18	22	2.9	50	2.9	Lung Tumor
908F	101	0.0073	14	72	0.049	0.56	0.93	Unknown
778M	102	0.065	11	85	1.3	1.0	0.52	Pulmonary Thromboembolism
812M	103	0.288	15	36	29	16	2.2	Lung Tumor
814F	104	0.054	49	33	4.1	10	1.6	Lung Tumor
840F	107	0.389	17	35	5.8	37	2.0	Lung Tumor
777M	109	0.392	11	52	7.8	24	1.7	Lung Tumor
857M	109	0.333	20	39	9.4	27	2.4	Lung Tumor
898F	111	0.333	10	34	28	21	2.1	Lung Tumor
899F	113	0.0066	7.5	87	0.14	0.27	1.6	Hemangiosarcoma, Heart
697M	114	0.141	15	64	8.1	9.9	1.4	Cardiac Insufficiency
909M	115	0.444	16	46	11	25	1.2	Lung Tumor
824F	116	0.178	21	75	0.50	2.3	0.70	Pneumonia
891M	116	0.0023	11	84	0.064	0.48	1.5	Septicemia
836M	117	0.333	12	53	15	7.3	0.97	Lung Tumor
794M	120	0.397	13	33	14	31	3.5	Pituitary Tumor

(a) Includes tracheobronchial, mediastinal and sternal lymph nodes

(b) Includes hepatic, splenic and mesenteric lymph nodes

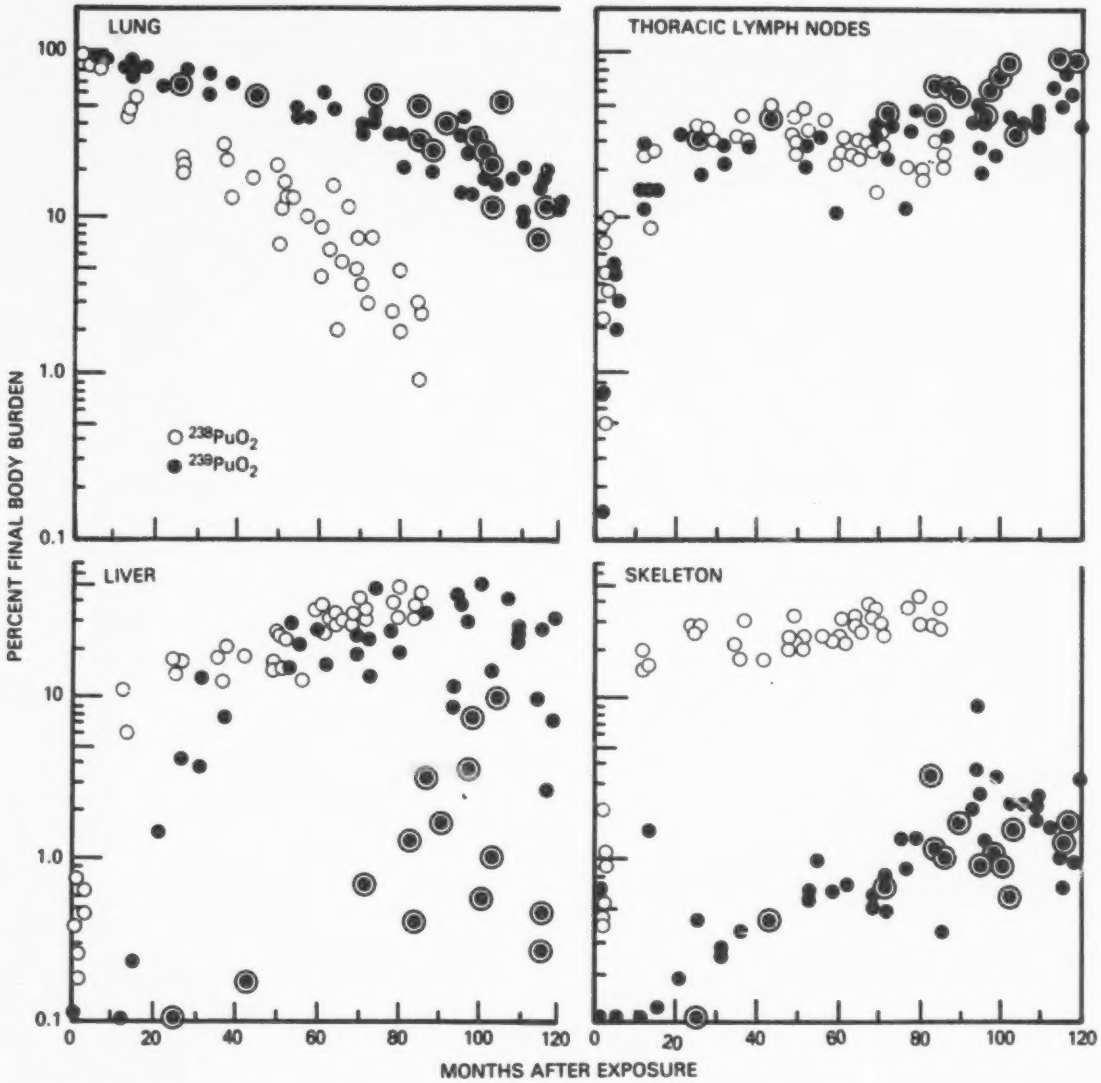

FIGURE 1. Tissue Distribution of Plutonium in Beagles After Inhalation of PuO$_2$ (◉ – Low-Dose Level ^{239}PuO$_2$ Dogs).

cytopenia. Livers of the dogs euthanized during the 4- to 10-yr postexposure period showed moderate, diffuse, centrilobular congestion. Liver cells in these areas contained fine, granular, yellow pigment resembling lipofuscin, and were frequently vacuolated. Focal aggregation of vacuolated, lipofuscin-containing cells in the sinusoids was associated with alpha stars on autoradiographs.

Lymphopenia developed after inhalation of ^{239}PuO$_2$ in dose level groups with mean initial alveolar depositions of 79 nCi or more (Figure 2). Through 115 mo after exposure, mean lymphocyte values were significantly lower ($P < 0.05$-0.01) for Dose Level Groups 3 and 4 than for the control group. The reduction in lymphocytes was dose-related, both in time of appearance and magnitude. Over the course of this study, there has been a slight age-related decrease in mean lymphocyte values of control dogs. In addition, mean lymphocyte concentrations in Groups 3 and 4 have tended to increase, making the differences between control dogs and these groups less significant than previously.

TABLE 4. Summary of Lesions in Dogs Euthanized During the 10-yr Period After Inhalation of $^{239}\text{PuO}_2$.

Dose Group	No. Dogs/ Group	No. Dead Dogs/ Group	Number of Dogs/Lesion Associated with Death													
			Radiation Pneumonitis	Lung Tumor	Malignant Lymphoma	Hemangiosarcoma	Reticulum Cell Sarcoma	Pituitary Tumor	Ovarian Tumor	Oral Tumor	Pneumonia	Thromboembolism	Septicemia	Epilepsy	Pyometra	Cardiac Insufficiency
6	8	8	7	1												
5	21	21	1	20												
4	22	10		6			1	1			2					
3	20	4		1								1			1	1
2	21	2							1					1		
1	24	4			1	1					1		1			
Control	20	1								1						

At mean alveolar depositions of 3.5 and 22 nCi, lymphocyte values were within ranges observed in control dogs. A reduction in total leukocytes was evident in the higher dose groups, which were also lymphopenic. No effects have been observed on red-cell parameters following $^{239}\text{PuO}_2$ inhalation.

Serum chemistry assays have been performed to detect organ-specific damage from plutonium that translocated from lung to extrapulmonary sites. No consistent, dose-related alterations have occurred in serum constituents (glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, alkaline phosphatase, urea nitrogen, and serum protein fractions) of dogs exposed to $^{239}\text{PuO}_2$.

Complete physical examinations were conducted on 90 $^{239}\text{PuO}_2$ dogs at 9 to 10 yr after exposure. Table 5 lists, by category and organ system, findings that are considered abnormal. Most physical abnormalities were evenly distributed among the six treatment groups; in some systems, differences appear between control and exposed groups. Incidences of cardiovascular abnormalities were higher (25%, 36% and 100%, respectively) in treatment Groups 3 through 5, compared to controls (16%). Respiratory abnormalities were detected only in exposed dogs. Only exposed dogs had testicular abnormalities or vaginal polyps.

Thyroid hormone determinations (T_3,T_4, radioimmunoassay performed by a commercial veterinary laboratory) were performed on 87 $^{239}\text{PuO}_2$-exposed dogs at 9 to 10 yr after exposure. Sixty-three dogs were euthyroid, 15 were hypothyroid; and 9 dogs with borderline values were classified as suspect (Table 6). Those dogs classified as suspect will be further tested through TSH-stimulation tests to confirm or deny the presence of thyroid dysfunction in them.

During the first 7½ yr following exposure to $^{238}\text{PuO}_2$, all of the dogs in the highest-level dose group, ten dogs in Dose Level Group 5, one dog in Group 4, and one dog in Dose Level Group 2 were euthanized when death was imminent. Twenty-one were sacrificed for comparison of plutonium tissue distribution. Table 7 and Figure 1 show the causes of death and the distribution of ^{238}Pu in the tissues of these animals. One 97-mo-old control dog died due to pyometritis.

Of the 25 euthanized dogs, 16 were killed due to bone tumors (osteosarcoma), three due to lung tumors, three due to Addison's disease, one due to hemangiosarcoma in the heart, one due to pneumonia and one due to paralysis. Eight of the dogs euthanized due to bone tumors also had lung tumors; two also had Addison's disease. Eight of the 16 bone tumors were in vertebrae; two in femurs, two in ribs, two in the scapulas, one in the pelvis and one in the tibia. Table 8 summarizes, by dose-level group, mortality and lesion associated with death through 7½ yr after exposure.

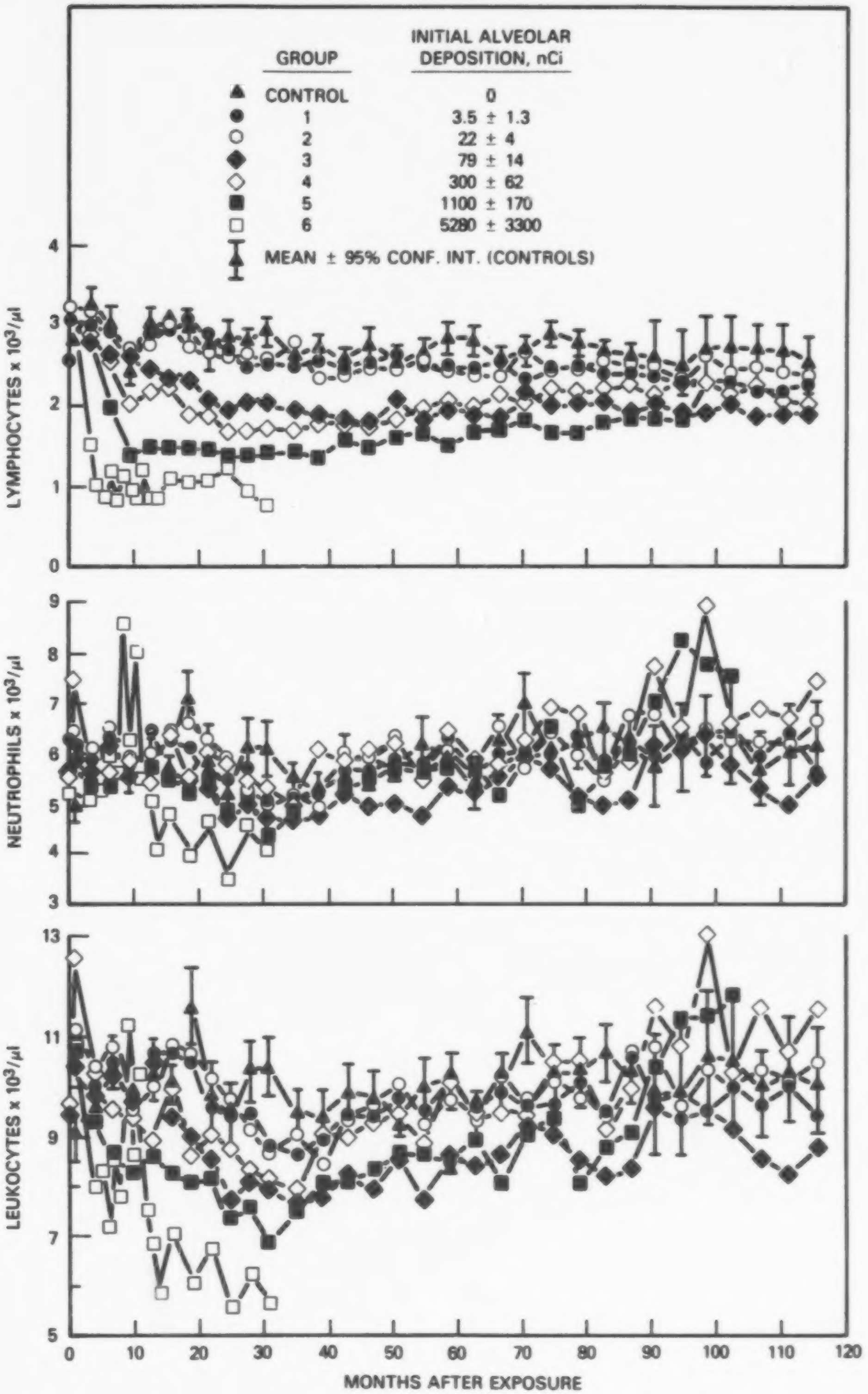

FIGURE 2. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{239}PuO_2$.

TABLE 5. Summary (by Numbers of Dogs) of Abnormal Physical Findings in $^{239}PuO_2$-Exposed Dogs 9 to 10 yr After Exposure.

Dose Group	No. Dogs/ Group	General		Skin & Subcutis			Oral Cavity			Eyes				Cardiovascular System	Respiratory system	Genitourinary System			Lymphoreticular System	
		Body Weight Loss	Poor Condition	Skin	Haircoat	Tumors	Gums	Teeth Arcade	Dental Formula	Cornea	Cataracts	Nuclear Sclerosis	Retina, Degeneration	Cardiac Abnormalities	Auscultation Abnormalities	Testes	Prostate	Vaginal Canal (polyps)	Lymphoreticular System	Tonsils
5	1	1	1		1	1			1					1	1	1				
4	14	3	3	2	5	9	8	2	12		2	10	2	5	4	4	4	2	2	
3	16	4		3	4	9	8	3	14		4	8	6	4	1	2	3	3	4	2
2	19	1	1		6	13	7	2	12		3	14	7	1			7	1		3
1	21	6	2	2	5	18	10	1	16	1	1	13	5	2	2	6	5	4	4	1
Control	19	3	2	4	5	16	7	3	19	1	4	13	10	3			6		6	1
	90																			

TABLE 6. Results of Thyroid Function Testing in $^{239}PuO_2$-Exposed Dogs 9 to 10 yr After Exposure.

Dose Group	No. Dogs/ Group	Number of Dogs		
		Euthyroid	Hypothyroid	Suspect
5	1		1	
4	12	7	4	1
3	16	14		2
2	19	17	2	
1	20	12	4	4
Control	19	13	4	2
Total	87	63	15	9

The lung tumors were classified as bronchiolar-alveolar carcinomas in eight dogs, bronchiolar-alveolar adenoma in one dog, and adenosquamous carcinoma in one dog. In one dog, three lung tumor types were observed: bronchiolar-alveolar, adenocarcinoma and fibrosarcoma. Lung tumor metastases were not observed. Bone tumor metastases were found in the lungs of five dogs; and in one dog, the bone tumor metastasized to liver, lung, and tracheobronchial lymph nodes. The five dogs with Addison's disease had adrenal cortical atrophy.

In addition to the lesions associated with the cause of death, lesions in the lungs of the high-dose-level dogs included focal alveolar histocytosis, alveolitis, alveolar epithelial cell hyperplasia, alveolar emphysema, pleural fibrosis, and interstitial fibrosis. Numerous alpha stars were observed, mainly in foci of fibrosis, and single alpha tracks were scattered throughout sections in foci of alveolar histocytosis and in alveolar septa. The tracheobronchial and mediastinal lymph nodes were completely obliterated by necrosis and scarring, associated with high concentrations of plutonium observed as alpha stars. Similar but less severe lesions were seen in the hepatic lymph nodes. There were extensive alterations in bone, including multiple areas of focal atrophy of bone; endosteal, trabecular and peritrabecular bone fibrosis; and osteolysis of cortical, endosteal, and trabecular bone. One dog had lesions of secondary hypertrophic osteoarthropathy. Radioactivity in the bone was present as single tracks, generally scattered throughout the bone, cartilage, and bone marrow. The liver contained foci of hepatocellular fatty change, where small clusters of single tracks were seen. There was also mild, focal, nodular hyperplasia of hepatocytes. Elevated serum glutamic pyruvic transaminase levels, suggestive of liver

TABLE 7. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{238}PuO_2$.

Dog Number	Time After Exposure, Mo	Final Body Burden, μCi	Percent of Final Body Burden: Lungs	Thoracic Lymph Nodes[a]	Abdominal Lymph Nodes[b]	Liver	Skeleton	Cause of Death
1032M	0.25	0.150	97	0.34	0.20	1.7	0.16	Sacrifice
921F	1	0.0044	93	0.65	0.04	0.38	2.1	Sacrifice
930F	1	0.052	99	0.63	0.01	0.07	0.35	Sacrifice
931F	1	0.347	96	1.9	0.01	0.05	0.36	Sacrifice
929F	2	0.017	91	7.5	0.002	0.26	0.58	Sacrifice
932F	2	0.382	96	2.5	0.01	0.18	0.39	Sacrifice
923F	2	0.0023	88	9.4	0.03	0.09	0.44	Sacrifice
925M	3	0.0064	91	4.1	0.04	0.04	1.2	Sacrifice
926M	3	0.078	87	11	0.23	0.65	1.1	Sacrifice
934M	3	0.902	92	4.8	1.7	0.45	0.95	Sacrifice
1318M	12	0.030	45	27	0.08	10	15	Sacrifice
1319M	12	0.077	41	26	0.03	11	20	Sacrifice
1214M	13	0.014	52	9.2	0.32	6.2	16	Sacrifice
1310M	25	0.026	19	36	0.08	15	28	Sacrifice
1317M	25	0.041	20	33	0.16	17	26	Sacrifice
1315M	25	0.047	22	31	0.04	17	28	Sacrifice
1191F	35	0.658	26	32	0.13	18	22	Pneumonia
1215M	36	0.011	21	43	0.17	13	21	Sacrifice
1311M	37	0.036	13	31	0.22	21	32	Sacrifice
994F	42	5.024	17	45	0.50	18	18	Addison's Disease
970F	48	0.0022	20	34	0.36	16	24	Sacrifice
1312M	49	0.035	6.8	29	0.26	25	35	Sacrifice
1143M	49	6.331	11	43	2.0	15	22	Bone Tumor, Lung Tumor
1025M	50	10.033	16	27	7.1	24	23	Lung Tumor
1064M	51	8.427	13	48	1.9	15	20	Bone Tumor, Lung Tumor
1175F	52	3.641	14	31	0.08	25	26	Lung Tumor
1079M	56	2.182	9.8	40	4.3	13	25	Addison's Disease
1096F	59	1.204	4.3	22	2.7	36	24	Addison's Disease
1189M	60	0.044	8.9	25	0.16	37	25	Sacrifice
1115F	61	1.534	5.0	32	2.3	26	33	Bone Tumor
1162F	61	3.663	12	32	5.9	21	25	Bone Tumor, Addison's Disease
1009M	62	4.360	15	25	2.4	31	23	Lung Tumor
974F	64	1.465	5.1	24	5.9	33	29	Bone Tumor
1092M	65	1.515	2.1	26	9.1	29	30	Bone Tumor
975F	66	3.749	11	30	2.1	28	25	Bone Tumor, Lung Tumor
1042F	69	1.494	4.7	25	2.9	32	33	Bone Tumor, Lung Tumor
1037M	69	2.417	7.1	27	7.8	28	27	Bone Tumor
1027M	70	2.546	3.8	15	7.0	40	31	Bone Tumor, Lung Tumor
1006F	72	2.826	7.5	30	3.4	29	26	Bone Tumor, Lung Tumor
1057M	72	1.748	3.0	35	2.2	33	24	Bone Tumor
1082M	78	0.0083	2.4	20	0.31	40	34	Paralysis
1081M	80	0.361	4.6	15	0.48	47	29	Hemangiosarcoma, Heart
1058F	80	1.000	2.0	18	4.4	31	41	Bone Tumor, Adrenal Tumor
1002M	84	1.786	2.9	31	2.0	31	28	Bone Tumor, Lung Tumor
1109F	86	0.885	0.93	23	4.0	34	35	Bone Tumor, Addison's Disease, Lung Tumor
1218F	86	0.678	2.7	23	4.1	42	25	Bone Tumor

(a) Includes tracheobronchial, mediastinal and sternal lymph nodes

(b) Includes hepatic, splenic and mesenteric lymph nodes

damage, were observed in the high-dose-level dogs.

Dose-related lymphopenia was observed in groups with mean alveolar ^{238}Pu deposition of 77 to 5200 nCi (Figure 3). The lymphocyte depression was more pronounced, both in magnitude and earlier appearance, than in dogs exposed to similar doses of $^{239}PuO_2$. However, lymphocyte values in the $^{238}PuO_2$-

TABLE 8. Summary of Lesions in Dogs Euthanized During the 7.5-yr Period After Inhalation of $^{238}PuO_2$.

			Number of Dogs/Lesion Associated with Death									
Dose Group	No. Dogs/ Group	No. Dead Dogs/ Group	Lung Tumor	Bone Tumor	Bone Tumor & Lung Tumor	Bone Tumor & Addison's Disease	Bone Tumor, Addison's Disease & Lung Tumor	Addison's Disease	Hemangiosarcoma, Heart	Pneumonia	Spinal Cord Degeneration	Pyometra
6	13	13	3	2	6	1		1				
5	20	10		5	1		1	2		1		
4	20	1							1			
3	22	0										
2	21	1									1	
1	20	0										
Control	20	1										1

exposed dogs tended to increase sooner after reaching a minimum than in $^{239}PuO_2$-exposed dogs. Mean lymphocyte concentrations in Group 3 dogs were not significantly different from values of control dogs over the past year. The difference between $^{238}PuO_2$ and $^{239}PuO_2$ in effects on lymphocytes may be related to the early translocation of ^{238}Pu to bone and bone marrow, with accompanying irradiation of those lymphocytes as well as in the lungs and tracheobronchial lymph nodes. As bone remodeling occurs, bone marrow irradiation may decrease as the ^{238}Pu is buried deeper in compact bone. This speculation is supported by the increase in neutrophil values of Group 5 dogs over the past 2 yr. As with ^{239}Pu, lymphocyte values in the two lowest exposure groups (2.3 and 18 nCi) were not different from control values. A dose-related reduction in total leukocytes was evident, primarily due to lymphopenia, except in Groups 5 and 6, in which neutropenia was observed. No difference in monocyte values was seen in relation to dose levels. A significant and progressive reduction in eosinophils was evident only in Group 6 dogs following $^{238}PuO_2$ inhalation. No chronic effects have been observed in red-cell parameters.

Lymphopenia was the earliest observed effect after inhalation of either $^{239}PuO_2$ or $^{238}PuO_2$, occurring after deposition of ~80 nCi plutonium in the lungs. On a concentration basis, the 80-nCi dose level is about 40 times the 16-nCi maximum permissible human lung deposition, based on 0.3 rem/wk to the lung.

In serum chemistry assays of $^{238}PuO_2$ dogs, glutamic pyruvic transaminase values were higher than those of the control group only in Group 6 dogs (5200 nCi) after about 46 mo following exposure. Elevations in this "liver-specific" enzyme are consistent with histopathologic findings and radiochemical analyses of ^{238}Pu translocation to the liver. Alkaline phosphatase was elevated in some of the dogs with bone tumors.

Complete physical examinations were performed on 106 $^{238}PuO_2$ dogs at 6.5 to 7.5 yr after exposure; Table 9 lists abnormal findings by organ system and category. In general, the exposed dogs were in poorer overall condition than controls. This evaluation (unrelated to dose) was reflected by the more frequent occurrence of weight loss, abnormalities of the skin and haircoat, of the oral cavity, and of the lymphoreticular system.

At 6½ to 7½ yr after exposure, the fraction of the final body burden in the lungs of the ^{238}Pu-exposed dogs was about 3%, compared to 26% in the ^{239}Pu-exposed dogs (Figure 1). At that time, ~22% of the ^{238}Pu was in the thoracic lymph nodes, compared to ~55% of the ^{239}Pu. Livers of the ^{238}Pu dogs contained ~35% of the Pu burden, compared to ~25% in the livers of the ^{239}Pu dogs. About 32% of the final

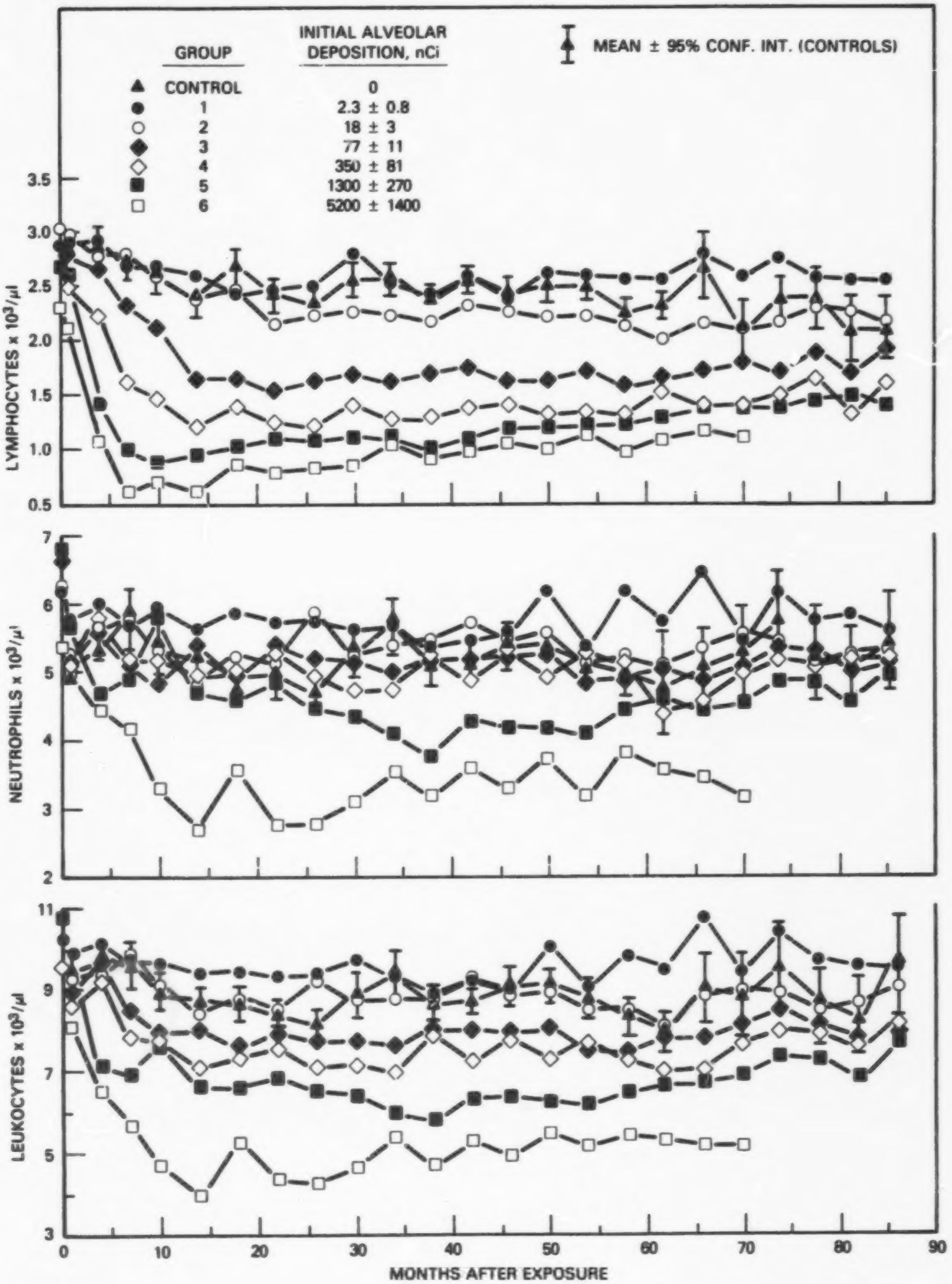

FIGURE 3. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{238}PuO_2$.

TABLE 9. Summary (by Numbers of Dogs) of Abnormal Physical Findings in $^{238}PuO_2$-Exposed Dogs 6.5 to 7.6 yr After Exposure.

Dose Group	No. Dogs/ Group	General	Body Weight Loss	Poor Condition	Skin & Subcutis	Skin	Haircoat	Tumors	Oral Cavity	Gums	Teeth Arcade	Dental Formula	Eyes	Cornea	Cataracts	Nuclear Sclerosis	Retina, Degeneration	Cardiovascular System	Cardiac Abnormalities	Respiratory system	Auscultation Abnormalities	Genitourinary System	Testes	Prostate	Vaginal Canal (polyps)	Lymphoreticular System	Lymphoreticular System	Tonsils
5	9		1			1		7		7	1	4				2	5						4	3	1		1	
4	21			1		3	2	21		9	8	8			2	9	4		3		1		2	2			2	2
3	18		3				1	14		11	7	6				10	7						3	2	5			
2	20		4			1		16		12	7	7			4	12	6		3				6	5	2		2	2
1	19		1				1	19		10	7	5				10	6		1				2	4	1		3	3
Control	19							14		7	6	4			2	7	9		2		1		5	2	3			1
	106																											

body burden was in the skeletons of the ^{238}Pu-exposed dogs, at that time, compared to less than 2% in the ^{239}Pu-exposed dogs.

Tissue distribution of ^{238}Pu in low-dose-level dogs did not differ from that in high-dose-level dogs.

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• Inhaled Plutonium Nitrate in Dogs

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The major objective of this project is to determine dose-effect relationships of inhaled plutonium nitrate in dogs to aid in the prediction of health effects of accidental exposure in man. For lifespan dose-effect studies, beagle dogs were given a single inhalation exposure to $^{239}Pu(NO_3)_4$, in 1976 and 1977. The earliest biological effect was on the hematopoietic system; as described in previous Annual Reports, lymphopenia and neutropenia occurred at the two highest dose levels. We have also observed radiation pneumonitis, lung cancer, and bone cancer at the highest dose levels.

The skeleton and liver are generally considered the critical tissues after inhalation of "soluble" plutonium (as in the case of plutonium nitrate), on the assumption that the plutonium will be rapidly translocated from the lung to skeleton and liver. In several rodent studies, however, inhalation of "soluble" plutonium has resulted in lung tumors as well as skeletal tumors. Lifespan studies are necessary to evaluate the complex interactions between tissues and organ systems directly or indirectly impaired by lower levels of exposure. Beagle dogs were chosen to correlate relative risks, determined in other studies, with different forms and routes of exposure to plutonium.

Six dose groups (105 dogs) were exposed, in 1976 and 1977, to aerosols of $^{239}Pu(NO_3)_4$ for lifespan observations (Table 1). In addition, 20 dogs were exposed to nitric acid aerosols as vehicle controls, 25 dogs were exposed to aerosols of $^{239}Pu(NO_3)_4$ for periodic sacrifice to study plutonium metabolism and the pathogenesis of developing lesions, seven dogs were selected as controls for periodic sacrifice, and 20 dogs were selected as untreated controls for lifespan observations. The dogs were exposed in aerosol chambers, using techniques described in previous reports. The Appendix (following the entire Annual Report) shows the current status of each dog on these experiments.

TABLE 1. Lifespan Dose-Effect Studies with Inhaled $^{239}Pu(NO_3)_4$ in Beagles[a].

Dose Level Group	Number of Dogs		Initial Alveolar Deposition[b]	
	Male	Female	nCi[c]	nCi/g Lung[c]
Control	10	10	0	0
Vehicle	10	10	0	0
1	10	10	2 ± 2	0.02 ± 0.02
2	10	10	8 ± 4	0.06 ± 0.04
3	10	10	56 ± 17	0.5 ± 0.2
4	10	10	295 ± 67	2 ± 0.8
5	10	10	1709 ± 639	14 ± 6
6	3	2	5445 ± 1841	47 ± 17

[a] Exposed in 1976 and 1977

[b] Estimated from external thoracic counts at 2 weeks post-exposure and estimated lung weights (0.011 x body weight)

[c] Mean ± standard deviation

The initial deposition and early clearance of inhaled $^{239}Pu(NO_3)_4$ aerosols were discussed in previous Annual Reports. The fraction of plutonium in the lung decreased to approximately 2% of the final body burden in dogs surviving 4 yr or more (Table 2). There was early translocation to the liver and skeleton, with an average of 34% and 61%, respectively, of final body burden present in these tissues in dogs surviving 4 yr or more. Only minimal amounts were translocated to thoracic or abdominal lymph nodes. This was in contrast to dogs that inhaled $^{239}PuO_2$, in which a considerable amount translocated to the thoracic lymph nodes, but only minimal amounts translocated to liver or skeleton at these time periods. In a pilot study reported previously (Annual

TABLE 2. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}Pu(NO_3)_4$.

Dog Number	Time After Exposure, mo	Final Body Burden, µCi	Percent of Final Body Burden: Lungs	Thoracic Lymph Nodes[a]	Abdominal Lymph Nodes[b]	Liver	Skeleton	Cause of Death
1359M	0.1	0.080	90.50	0.15	0.06	2.46	3.20	Sacrifice
1375F	0.1	0.073	89.61	0.14	0.01	0.97	4.68	Sacrifice
1407F	0.1	0.092	51.87	0.41	0.13	10.99	18.70	Sacrifice
1389M	0.5	0.053	24.07	0.38	0.08	41.28	26.21	Sacrifice
1390M	0.5	0.051	24.62	0.32	0.11	20.05	44.45	Sacrifice
1445F	0.5	0.057	26.42	0.32	0.11	21.28	44.73	Sacrifice
1329F	1	0.485	70.05	0.16	0.04	8.28	18.79	Sacrifice
1346M	1	0.902	76.81	0.32	0.03	10.45	10.30	Sacrifice
1347F	1	0.699	71.71	0.36	0.08	9.33	14.09	Sacrifice
1336M	1	0.032	71.38	0.22	0.05	5.72	19.73	Sacrifice
1341F	1	0.022	64.43	0.29	0.10	12.92	18.63	Sacrifice
1344F	1	0.052	58.68	0.25	0.04	21.87	16.09	Sacrifice
1335M	1	0.001	19.52	0.07	0.06	6.68	25.04	Sacrifice
1339F	1	0.001	19.08	0.11	0.08	20.92	45.47	Sacrifice
1351M	1	0.002	40.68	1.22	0.09	17.09	28.89	Sacrifice
1522F	3	0.059	54.68	0.57	0.10	11.52	28.24	Sacrifice
1529F	3	0.049	51.68	0.40	0.07	18.48	23.74	Sacrifice
1539M	3	0.072	52.45	0.31	0.05	18.58	25.03	Sacrifice
1564F	12	0.037	18.00	1.27	0.14	33.53	42.63	Sacrifice
1571F	12	0.051	22.37	1.47	0.11	28.76	42.91	Sacrifice
1588M	12	0.053	13.14	0.40	0.12	35.85	46.18	Sacrifice
1424M	14	4.625	33.10	1.43	0.16	26.49	36.88	Radiation Pneumonitis
1518F	16	4.025	18.99	0.94	0.18	29.51	47.88	Radiation Pneumonitis
1510F	17	4.048	22.00	1.15	0.05	20.71	52.00	Radiation Pneumonitis
1420M	25	1.616	16.51	0.86	0.20	7.77	70.06	Radiation Pneumonitis
1471M	34	1.375	9.25	0.73	0.12	26.92	58.34	Radiation Pneumonitis
1518M	42	1.880	6.87	0.24	0.07	21.34	67.51	Radiation Pneumonitis + Lung Tumor
1512M	42	2.136	4.31	0.60	0.08	49.93	42.66	Bone Tumor
1508M	43	1.730	3.24	0.62	0.08	41.53	52.70	Bone Tumor
1459F	51	1.567	4.40	0.15	0.12	30.86	61.41	Radiation Pneumonitis + Lung Tumor
1492F	52	1.202	2.31	0.20	0.17	27.02	66.38	Bone Tumor
1502F	54	3.113	0.80	0.39	0.09	33.33	62.51	Bone Tumor, Lung Tumor
1485F	55	1.052	0.82	0.35	0.07	31.13	63.94	Bone Tumor
1387F	55	0.167	1.41	0.22	0.12	45.48	49.10	Bone Tumor
1429M	59	--	--	--	--	--	--	Bone Tumor, Lung Tumor

(a) Includes tracheobronchial, mediastinal and sternal lymph nodes
(b) Includes hepatic, splenic and mesenteric lymph nodes

Report, 1979), $^{238}Pu(NO_3)_4$ translocated more rapidly to liver and skeleton than did $^{239}Pu(NO_3)_4$, but both reached a similar plateau at 1 yr after exposure.

The earliest observed biological effect was on the hematopoietic system: lymphopenia occurred at the two highest dose levels at 4 wk after exposure to $^{239}Pu(NO_3)_4$. The results of these continuing evaluations through 54 mo postexposure are shown in Figure 1. Total leukocyte concentrations were reduced significantly in the two highest dose groups, i.e., Group 5

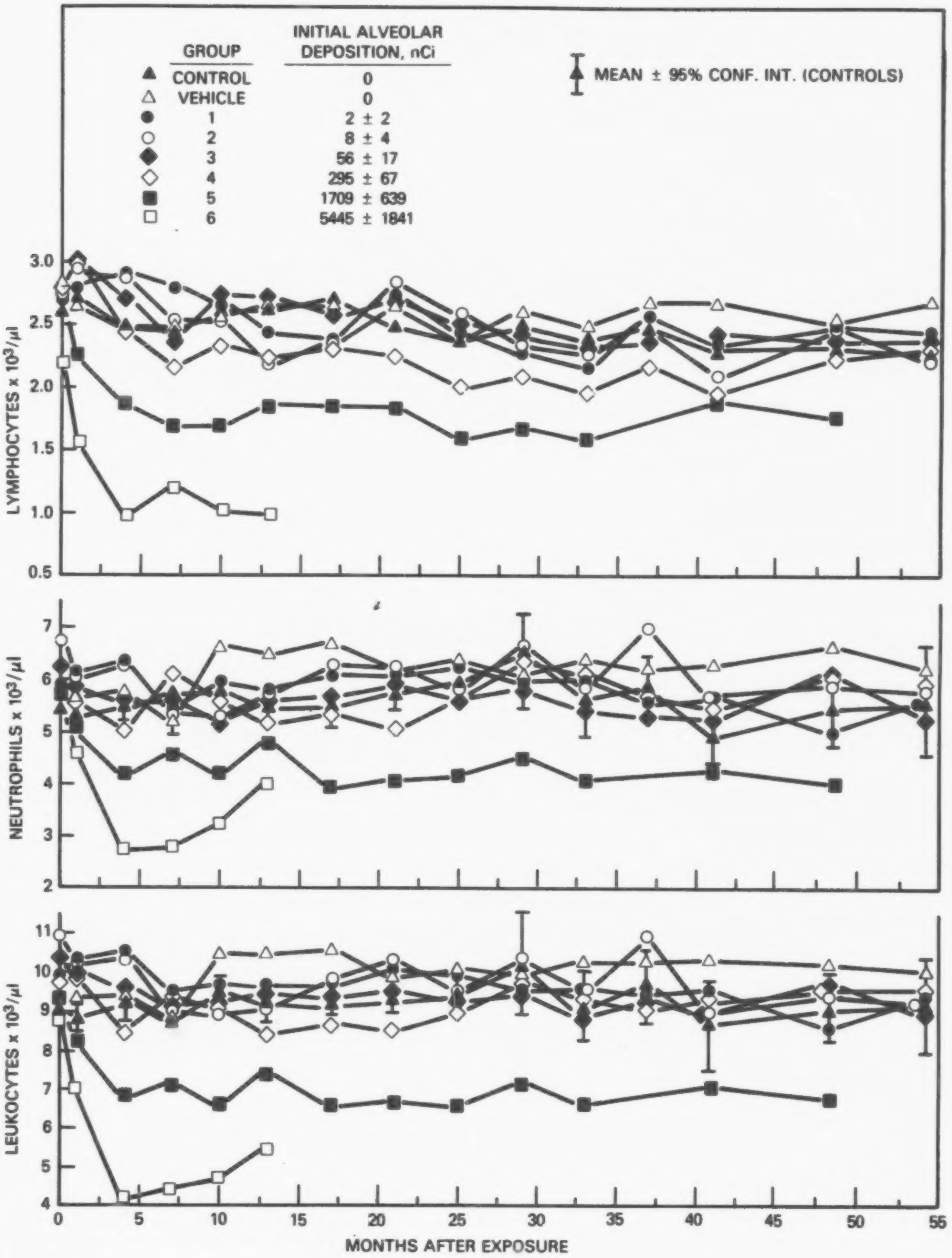

FIGURE 1. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{239}Pu(NO_3)_4$.

(mean initial alveolar deposition, ~1700 nCi), and Group 6 (~5500 nCi). Numbers of leukocytes in Group 4 dogs (~300 nCi) tended to be consistently (but not significantly) lower than those from control dogs. The reduction in white cells in Groups 5 and 6 is due to an effect on most leukocyte types (neutrophils, lymphocytes, monocytes and eosinophils). Lymphocyte reductions differed significantly from values for controls only in Groups 5 and 6. This is in contrast to the effects of both $^{239}PuO_2$ and $^{238}PuO_2$, which significantly depressed lymphocyte concentrations by 21 mo after exposure to initial lung burdens of ~80 nCi or more. The lymphocytopenia at lower dose levels of plutonium oxides may be related to the more-extensive translocation of plutonium oxide to the tracheobronchial lymph nodes.

All five dogs at the highest dose level, and two of 20 dogs at the medium-high dose level, died from radiation pneumonitis 14 to 51 mo after exposure. Histopathologic examination of these dogs' lungs revealed interstitial fibrosis, alveolar epithelial hyperplasia, increased numbers of alveolar macrophages, occasional small emphysematous cavities and, at times, very small nodules of squamous metaplasia at the termini of respiratory bronchioles.

Small, multiple, bronchioloalveolar carcinomas occurred in two dogs with radiation pneumonitis and in two additional dogs euthanized because of osteosarcomas. Typically, these arose in subpleural areas in proximity to areas of interstitial fibrosis or small cavities communicating with bronchioles. They were composed of irregular proliferations of cuboidal epithelial cells, forming aggregates of epithelial cells extending into adjacent alveoli. No metastases or invasions of nonpulmonary parenchyma were observed.

Osteosarcomas were present in seven dogs euthanized 42 to 59 mo after exposure: six dogs from the Group 5 dose level and one dog at the Group 4 dose level. The osteosarcomas occurred singly, in humerus, pelvis, sacrum, cranium, cervical vertebra, thoracic vertebra, and lumbar vertebra. These dogs also had radiation pneumonitis, as described previously, as well as radiation osteosis. The osteosis was generally characterized by peritrabecular fibrosis, composed of relatively hypocellular collagen fibers, and was observed partially surrounding trabeculae in vertebrae, femora, and ribs.

Autoradiographs of liver sections from dogs euthanized 3 to 5 yr after inhalation exposure to the higher dose levels of $^{239}Pu(NO_3)_4$ were compared with liver sections from dogs exposed to levels of $^{239}PuO_2$ that yielded similar concentrations of plutonium in the liver at similar intervals after exposure. The autoradiographs showed that the nitrate-exposed dogs had >99% of plutonium activity in diffusely distributed single tracks (only rarely in alpha stars), whereas the oxide-exposed dogs had >99% of the plutonium activity concentrated in alpha stars (only rarely in single tracks). The difference in microdistribution and character of the alpha activity probably influenced the biological effect.

Serum enzyme assays have been performed throughout the postexposure period in an attempt to diagnose specific damage to liver and/or bone by plutonium translocated by the lung. Although periodic elevations occurred in mean values for glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, and alkaline phosphatase, there were no dose-related or dose-consistent elevations in these values. The periodic excursions in mean values were usually because of high values in one or two dogs at a particular sampling period, and occurred in all treatment groups, including controls.

• Inhaled Americium Dioxide

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This project includes experiments to determine the effects of Zn-DTPA therapy on the retention, translocation and biological effects of inhaled $^{241}AmO_2$.

Beagle dogs that received inhalation exposure to $^{241}AmO_2$ developed leukopenia, clinical chemistry changes associated with hepatocellular damage, and were euthanized due to respiratory insufficiency caused by radiation pneumonitis 120-131 days after pulmonary deposition of 22 to 65 μCi ^{241}Am. Eighty percent of the initial pulmonary-deposited ^{241}Am was retained in the dogs, with 30% in the liver, 30% in the skeleton, and 10% in the lungs. Another group of dogs that received inhalation exposure to $^{241}AmO_2$ and were treated daily with Zn-DTPA (diethylenetriamine pentaacetic acid trisodium zinc salt) had initial pulmonary deposition of 19 to 26 μCi ^{241}Am. These dogs did not develop respiratory insufficiency, and hematologic and clinical chemistry changes were less severe than in the non-DTPA-treated dogs. At sacrifice, 195 days after exposure, 4% of the initial pulmonary-deposited ^{241}Am was retained in the DTPA-treated dogs, with 2.5% in the lungs, 0.1% in the liver, and 1% in the skeleton.

To determine the effects of DTPA therapy on the retention, translocation, and biological effects of inhaled $^{241}AmO_2$, beagle dogs received a single nose-only inhalation exposure to $^{241}AmO_2$ and one-half of the dogs were treated with Zn-DTPA (diethylenetriamine pentaacetic acid trisodium zinc salt). Table 1 shows the experimental design. The dogs scheduled for sacrifice at 3, 10, 29, and 91 days after exposure were used for retention and translocation studies only. Those dogs scheduled for sacrifice at 270, 810, and 1600 days after exposure were used for retention, translocation, and biological effect studies.

TABLE 1. Experimental Design.

	Sacrifice Schedule, No. of Dogs						
Days After Exposure	3	10	29	91	270	810	1600
$^{241}AmO_2$	3	3	3	3	3	3	3
$^{241}AmO_2$ plus Zn-DTPA	3	3	3	3	3	3	3
Zn-DTPA							6
Control							6

The $^{241}AmO_2$ was prepared by calcining the oxalate at 750°C for 2 hr. Aliquots of the dry powder were suspended in double-distilled water adjusted to a pH of 10 with NH_4OH. Large particles were removed by sedimentation. The supernatant containing small particles was readjusted to a pH of 10 and nebulized. Aerosol particles were dried by heating an air dilution and were presented for nose-only inhalation exposure to the dogs. Aerosol particle size distribution ranged from 1.3 μm AMAD with a GSD of 2.32 to 2.18 μm AMAD with a GSD of 2.39. Electron microscopy showed the aerosol particles were clusters of primary particles ranging in diameter from 0.03 μm to 1 μm.

The beagle dogs ranged from 2.1 to 8.5 yr old and weighed 10.7 to 16.9 kg at exposure. DTPA-treated dogs were given 0.50 mmole Zn-DTPA in single daily subcutaneous injections beginning 1 hr after inhalation exposure to $^{241}AmO_2$.

The 3, 10, 29, and 91-day sacrifices were completed as scheduled. Six of the nine non-DTPA-treated dogs exposed to $^{241}AmO_2$ and scheduled for sacrifice during the 270- to 1600-day postexposure period were euthanized due to respiratory insufficiency 120-131 days after exposure. Three of the $^{241}AmO_2$-exposed, DTPA-treated dogs were sacrificed at 195 days after exposure to compare ^{241}Am tissue distribution with the euthanized dogs. The three remaining non-DTPA-treated and six DTPA-treated

$^{241}AmO_2$-exposed dogs plus the six DTPA-treated controls and six nontreated controls are remaining on the study.

Table 2 shows the initial ^{241}Am body burden obtained by summarizing the ^{241}Am content of all excreta and tissues. Initial pulmonary burden was estimated by subtracting the fecal excretion for the first 3 postexposure days from the initial body burden. During the 195-day postexposure period the final ^{241}Am body burden of the DTPA-treated dogs decreased to ~4% of the initial pulmonary burden compared to ~80% of the initial pulmonary burden retained in the non-DTPA-treated dogs. The liver and the skeleton of the DTPA-treated dogs decreased to <1% of the initial pulmonary burden compared to ~30% of the initial pulmonary burden in each tissue for the nontreated dogs (Figure 1). The lungs of the DTPA-treated dogs decreased to 2.5% of the initial pulmonary burden compared to 10% of the initial pulmonary burden in the nontreated dogs.

During the 195-day postexposure period the DTPA-treated dogs excreted ~96% of the initial pulmonary burden compared to 20% for the nontreated dogs. In the DTPA-treated dogs 73% of the initial pulmonary burden was excreted in the urine and 23% in the feces. In the nontreated dogs, 6% of the initial pulmonary burden was excreted in the urine and 14% in the feces.

The fraction of the daily ^{241}Am body burden (obtained by subtracting each day's ^{241}Am excretion from the previous day's body burden) excreted decreased from 5% at 3 days postexposure to 0.1% at 195 days postexposure in the DTPA-treated group, compared to 1% at 3 days postexposure, decreasing to 0.02% in the nontreated group (Figure 2). After 195 days of treatment the DTPA-treated dogs were excreting about five times more, as percent of their body burden, than the nontreated dogs.

Hematologic data are shown in Figure 3. The $^{241}AmO_2$-only exposed dogs had a modest reduction in mean erythrocyte values, which were significantly lower ($P < 0.05$) than in control dogs at 10 and 60 days after exposure. Erythrocyte values of dogs given DTPA after $^{241}AmO_2$ exposure were reduced as compared to control dogs, but the differences between these groups were not statistically significant. There were no significant differences in any hematologic parameters between dogs given DTPA only and the control dogs. Americium-only exposed dogs had significant reductions ($P < 0.05$ to 0.001) in mean total leukocyte, neutrophil, lymphocyte and platelet concentrations from 10 days through 9 months after exposure compared to values from control dogs. Reticulocyte concentrations were reduced at 30 and 60 days after $^{241}AmO_2$ exposure but the difference from control values was significant only at 30 days ($P < 0.01$). Americium-241 exposure followed by DTPA treatment resulted in a reduction of total leukocytes, neutrophils and lymphocytes at 30 days after $^{241}AmO_2$ exposure. At 2, 3, and 6 months after exposure only lymphocyte values were lower than in control dogs ($P < 0.05$ to 0.001). At 9 months after exposure lymphocyte values in these dogs were still lower than in control dogs, but the difference was not significant. DTPA treatment had an obvious "sparing" action on the hematologic effects of americium exposure. At 10 days through 6 months postexposure, dogs administered DTPA following $^{241}AmO_2$ exposure generally had significantly higher leukocyte, neutrophil, lymphocyte, and platelet concentrations than those not treated with DTPA ($P < 0.05$ to 0.001). At 9 months following exposure $^{241}AmO_2$-exposed, DTPA-treated dogs still had higher values than untreated dogs but the differences were not significant due to the small number of old dogs remaining in these two groups, i.e., 3 in the $^{241}AmO_2$-only exposed group and 6 in the group given DTPA after $^{241}AmO_2$ exposure.

The effects of americium exposure on selected serum chemistry parameters are shown in Figure 4. Affected parameters were those associated with hepatocellular damage, i.e., elevated serum alkaline phosphatase (SAP), glutamic pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase (SGOT), and reduced cholesterol levels. The high variability in SGPT values from the control dogs was due primarily to one animal that consistently had markedly elevated levels in this parameter. Mean SGPT values from $^{241}AmO_2$-exposed, non-DTPA-treated dogs were higher than those from control dogs starting at 3 mo after exposure, but the differences were not statistically significant due to the individual variability. Americium-only exposed dogs had significantly higher ($P < 0.05$ to 0.01) SAP levels at 10 days through 6 months postexposure. The mean value at 9 months was ~80% higher than in control dogs but was not significantly different between the groups due to the small number of dogs alive in the exposed group. Cholesterol values were lower in the americium-exposed dogs than in the controls at all sampling periods after exposure, and the difference was statistically significant at 1 and 2 mo after exposure ($P < 0.05$).

As was observed with the hematologic parameters, DTPA treatment reduced the effects of americium exposure. Serum

TABLE 2. Deposition and Retention of ^{241}Am in Dogs After Inhalation of $^{241}AmO_2$ and Treatment with Zn-DTPA.

						Initial Pulmonary Burden		Final Body Burden		
Dog Number	Sex	Time After Exposure, days	Sacrificed, S Euthanized, E	Treatment	Initial Body Burden, μCi	μCi	% Initial Body Burden	μCi	% Initial Body Burden	% Initial Pulmonary Burden
1669	M	3	S	DTPA	40.2	26.4	65.7	23.2	57.6	87.7
1161	F	3	S	DTPA	34.5	15.8	45.8	13.0	37.7	82.5
1295	F	3	S	DTPA	27.4	15.1	55.1	12.8	46.8	85.1
Mean					34.1	19.1	55.5	16.3	47.4	85.1
1673	M	3	S	0	34.4	12.3	35.8	12.3	35.7	99.7
1681	M	3	S	0	24.5	19.7	80.4	19.7	80.5	99.9
1290	F	3	S	0	29.8	21.2	71.1	21.1	70.7	99.6
Mean					29.5	17.7	62.4	17.7	62.3	99.7
1677	F	10	S	DTPA	34.1	17.4	51.0	12.5	36.6	71.7
1723	M	10	S	DTPA	35.9	21.2	59.1	14.7	40.8	69.2
1789	M	10	S	DTPA	31.4	19.0	60.5	11.2	35.8	59.1
Mean					33.8	19.2	56.8	12.8	37.7	66.7
1848	M	10	S	0	31.5	24.9	79.0	23.4	74.3	94.1
1671	F	10	S	0	40.1	14.9	37.2	14.2	35.4	95.3
1813	M	10	S	0	43.4	36.3	83.6	35.6	82.1	98.1
Mean					38.3	25.3	66.6	24.4	63.9	95.8
1850	M	29	S	DTPA	29.7	23.3	78.5	7.0	23.7	30.2
1795	M	29	S	DTPA	34.6	22.1	63.9	7.4	21.5	33.6
1797	M	29	S	DTPA	26.5	16.5	62.3	5.1	19.3	31.1
Mean					30.3	20.6	68.2	6.5	21.5	31.6
1807	M	29	S	0	33.5	26.0	77.6	24.8	74.1	95.2
1835	M	29	S	0	53.5	12.3	23.0	10.7	20.1	87.4
1163	M	29	S	0	54.4	39.1	71.9	36.3	66.6	92.8
Mean					47.1	25.8	57.5	23.9	53.6	91.8
1885	M	91	S	DTPA	29.6	14.8	50.0	2.1	7.0	13.9
1861	M	91	S	DTPA	35.6	20.5	57.6	3.7	10.3	17.9
1832	M	91	S	DTPA	17.9	5.5	30.7	0.5	3.0	9.8
Mean					27.7	13.6	46.1	2.1	6.8	13.9
1883	M	91	S	0	26.0	18.9	72.6	16.3	62.7	86.3
1805	M	91	S	0	25.2	14.9	59.1	12.7	50.6	85.7
1875	M	91	S	0	25.9	19.7	76.1	17.8	68.7	90.5
Mean					25.7	17.8	69.3	15.6	60.6	87.5
1884	M	195	S	DTPA	40.7	25.6	63.0	0.8	1.9	3.0
1896	M	196	S	DTPA	24.5	19.0	77.5	0.9	3.7	4.8
1876	M	196	S	DTPA	28.3	23.7	84.0	1.3	4.5	5.4
Mean					31.1	22.8	74.8	1.0	3.4	4.4
1812	M	120	E	0	77.1	64.9	84.1	59.1	76.6	91.2
1874	M	133	E	0	52.3	42.6	81.4	38.5	73.5	90.3
1781	M	163	E	0	62.5	42.8	68.5	31.2	49.9	72.9
1898	M	169	E	0	54.9	35.2	64.1	23.6	43.0	67.0
1856	M	191	E	0	27.9	21.5	77.0	14.3	51.2	66.5
1865	M	231	E	0	31.5	21.8	69.3	19.1	60.8	87.8

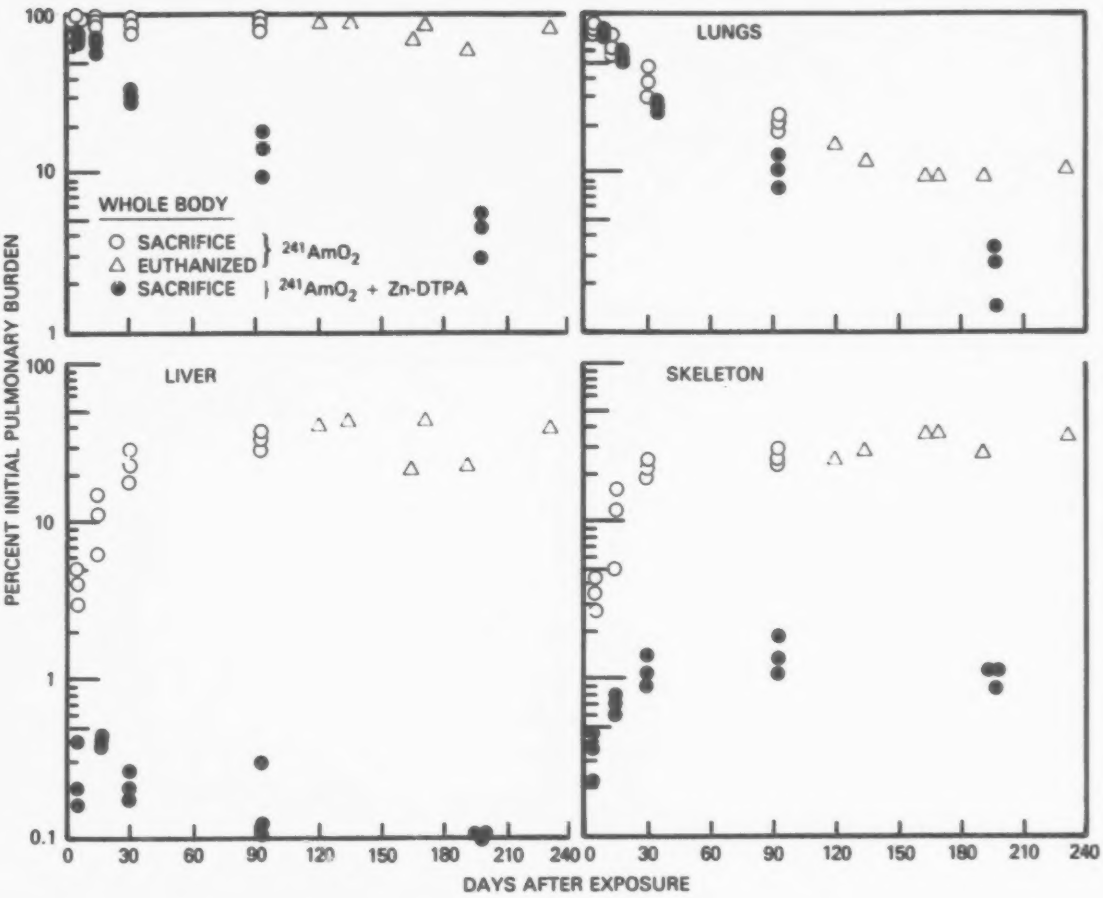

FIGURE 1. Retention of ^{241}Am in Dogs After Inhalation of $^{241}AmO_2$ and Treatment with Zn-DTPA.

chemistry parameters of dogs exposed to americium and administered DTPA were not significantly different from values obtained from the control dogs.

Dogs administered only DTPA each day for 9 mo had serum chemistry values similar to, and not significantly different, from those of the control group. This included evaluations of renal function, i.e., blood urea nitrogen and creatinine concentrations.

Six dogs exposed to $^{241}AmO_2$ that received no DTPA treatment died or were euthanized between 120 and 231 days after exposure. Death was due to respiratory insufficiency caused by radiation pneumonitis. The clinical signs in these dogs were characterized by progressive tachypnea, hypoventilation, anorexia, weight loss, and dehydration. Arterial blood gas analysis confirmed the presence of primary respiratory acidosis, hypercapnea, and hypoxia. Radiographic examination immediately prior to euthanasia revealed a diffuse, interstitial pattern of fibrosis consistent with radiation pneumonitis. Swabs taken from the trachea and main-stem bronchi at necropsy failed to demonstrate any evidence of bacterial involvement in the lungs of these dogs. None of the controls, DTPA-only-treated, or $^{241}AmO_2$-exposed dogs treated with DTPA have shown respiratory effects.

The decreased severity of hematological and clinical chemistry effects in the

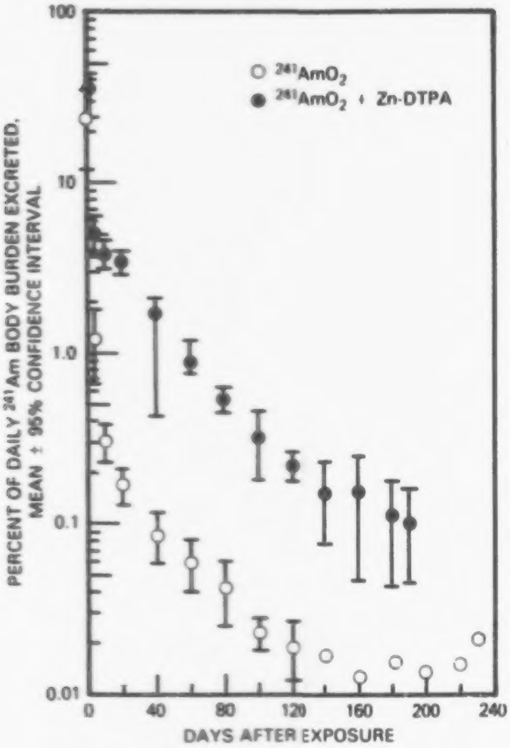

FIGURE 2. Excretion of ^{241}Am in Dogs After Inhalation of $^{241}AmO_2$ and Treatment with Zn-DTPA.

$^{241}AmO_2$-exposed, DTPA-treated dogs compared to the $^{241}AmO_2$-exposed, non-DTPA-treated dogs was consistent with the large decrease in retention of ^{241}Am in the skeleton and liver of the DTPA-treated dogs (Figure 1). Although DTPA treatment was less effective in decreasing lung retention than skeleton and liver retention, the $^{241}AmO_2$-exposed, DTPA-treated dogs have not developed respiratory insufficiency. The dose-effect relationships will be further evaluated to determine the influence of DTPA treatment on respiratory effects of inhaled $^{241}AmO_2$.

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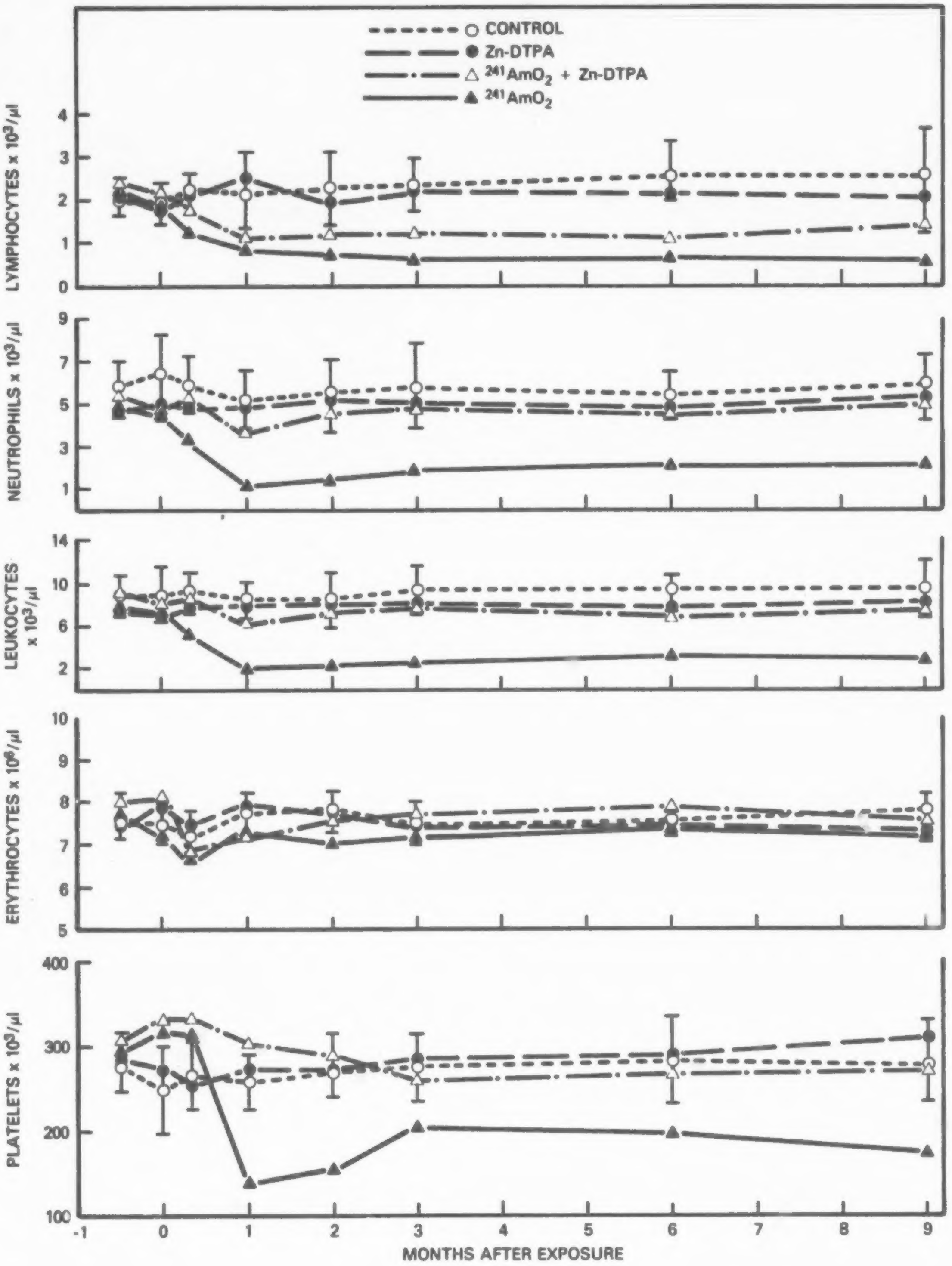

FIGURE 3. Mean Hematologic Values in Dogs After Inhalation of $^{241}AmO_2$ and Treatment with Zn-DTPA, Mean ± 95% Confidence Interval of Controls.

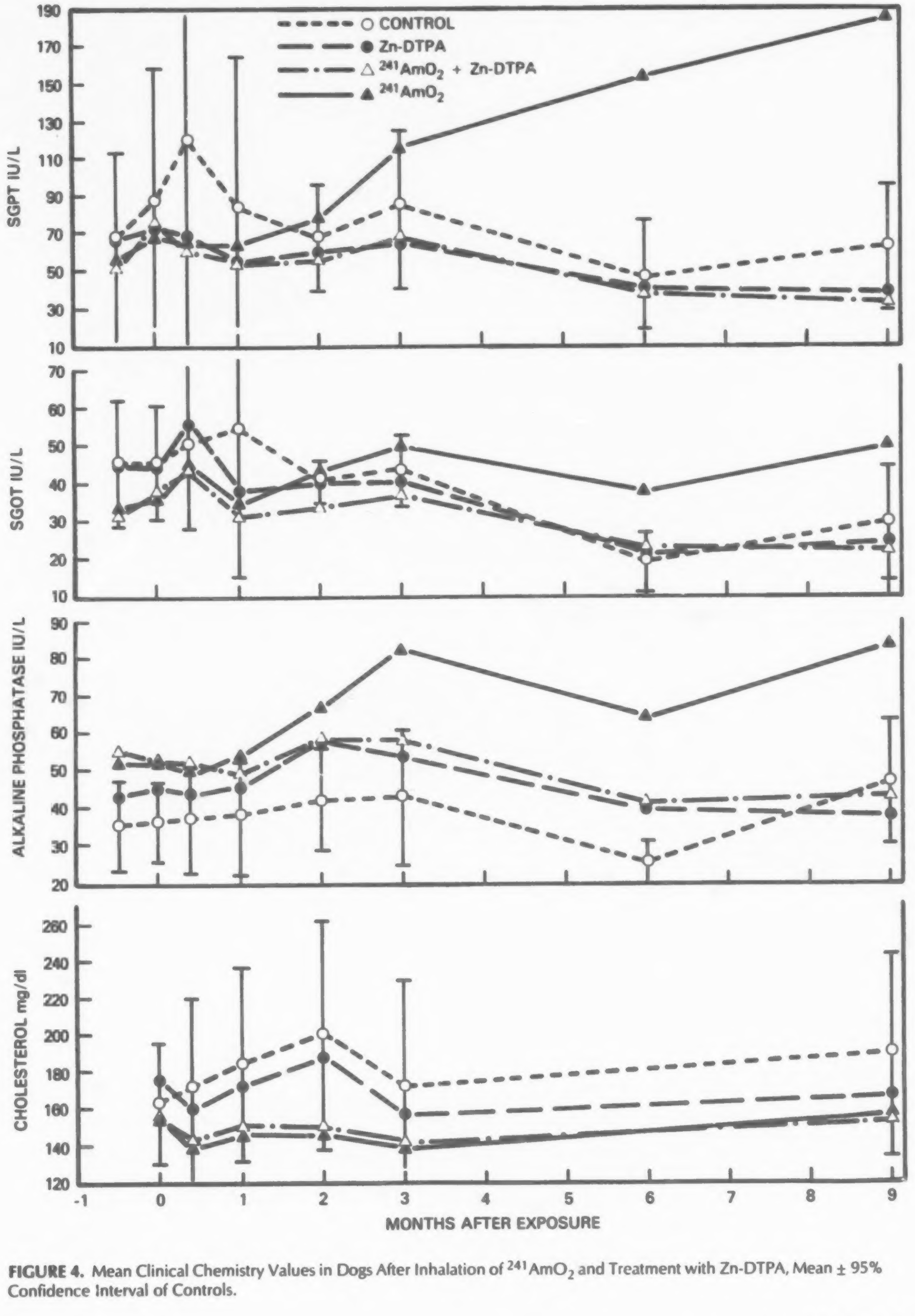

FIGURE 4. Mean Clinical Chemistry Values in Dogs After Inhalation of $^{241}AmO_2$ and Treatment with Zn-DTPA, Mean ± 95% Confidence Interval of Controls.

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• Inhaled Transuranics in Rodents

Principal Investigator: C. L. Sanders

Other Investigator: K. Rhoads

Pulmonary and skeletal carcinogenesis following inhalation of transuranic oxides is dependent upon spatial and temporal alpha dose-distribution patterns in the target tissues. Inhaled $^{241}AmO_2$ behaves similarly to inhaled $^{244}CmO_2$, resulting in lung and bone tumor incidences similar to those with $^{244}CmO_2$. Fractionated $^{244}CmO_2$ inhalation exposures, unlike fractionated exposures to inhaled $^{239}PuO_2$, increase the yield of lung and bone tumors. Immunological studies have not demonstrated a peripheral-blood lymphopenia, but have shown an altered humoral immune response. Vitamin C given to rats following inhalation of $^{239}PuO_2$ resulted in a nonsignificant decrease in lung-tumor incidence. An in vitro study of the radioresistance of alveolar macrophages showed that a dose of 10,000 rad is required to significantly reduce their bactericidal capability.

Because inhalation exposure is the most likely route of entrance for transuranics into people, it would be expected to yield the greatest carcinogenic risk. The project objectives are to characterize the deposition, retention, translocation and biological effects of inhaled transuranics in rodent models; to examine the influence of radiation dose, quality and dose-distribution in time and space on pulmonary and skeletal carcinogenesis; to examine anticarcinogenic factors that modify the carcinogenic process; and to examine the role of the humoral and cellular immune system in pulmonary carcinogenesis. Several lifespan studies have been completed in rats following inhalation exposure to transuranic oxides:

Inhalation Carcinogenesis of $^{241}AmO_2$

Wistar rats were given a single inhalation exposure to high-fired $^{241}AmO_2$ particles (AMAD, 0.75-1.39 μm) and examined over their life span. Of 311 rats, 259 were exposed to ^{241}Am for lifespan study, 31 rats were used for an early metabolism study of inhaled ^{241}Am, and 21 were unexposed controls.

About 55% of alveolar-deposited ^{241}Am was cleared from the lung with a half-life of 0.5 days, 37% with a half-life of 6.9 days, and 8% with a half-life of 580 days. Group mean lung doses ranged from 6 to 1500 rad. Significant early mortality due to radiation pneumonitis was seen only in the highest exposure group (>500 rad to lung). The frequency of lung tumors was 0 in controls (21 rats), 1% at lung doses of <10 rad (139 rats), 7% at 10-50 rad (86 rats), 0% at 50-100 rad (9 rats), 60% at 100-500 rad (10 rats) and 7% at >500 rad (15 rats). Only one bone tumor and one liver tumor were found in exposed animals.

The incidence of lung, bone and liver tumors following inhalation of ^{241}Am was compared to tumor incidence in a similar, earlier study (also in rats) with inhaled ^{244}Cm [Sanders et al., Radiat. Res. 76: 384 (1978)]. Tumor frequencies at comparable radiation doses were not significantly different (Tables 1 and 2), but the incidence of bone tumors may have been less with ^{241}Am than with ^{244}Cm.

Inhalation Carcinogenesis of Fractionated Exposures to $^{244}CmO_2$

Five groups of Wistar rats were included in this study: 1) unexposed shelf controls; 2) bottle-stressed, sham-exposed controls; 3) singly exposed at 70 days of age; 4) singly exposed at 267 days of age; and 5) exposed to 10 consecutive exposures at 3-wk intervals, starting at 70 days of age. Excretion of ^{244}Cm was also followed in groups 3, 4 and 5. All animals have died, and tissues have been examined for tumors.

Lung clearance of ^{244}Cm was estimated by the equation:

$$Y = 0.76e^{-1.28t} + 0.22e^{-0.059t} + 0.017e^{-0.0017t}.$$

The mean amount of ^{244}Cm in skeleton at 300 days after initial exposure to $^{244}CmO_2$ was ∼32 nCi for animals given a single exposure at 70 days of age, and 29 nCi for multiply exposed animals.

Survival of all groups exposed to ^{244}Cm was significantly decreased compared to that of unexposed control groups (Table 3). The incidence of lung tumors in rats singly exposed at 70 days of age was one-half the incidence seen in multiply exposed rats (9% vs 20%); metaplasias were also higher in multiply exposed rats (Table 4). One primary lung tumor was seen in a sham-exposed control. Bone tumors (osteochondrosarcoma) were seen twice as frequently in multiply exposed rats as in rats exposed at 70 days of age (24% vs 12%). Fewer lung tumors (no bone tumors)

TABLE 1. Comparison of Lung Dose-Response in Rats Exposed by Inhalation to $^{241}AmO_2$ and $^{244}CmO_2$ (Excluding 90 Rats Exposed to $^{241}AmO_2$ that had no Detectable ^{241}Am in Lungs at Death).

Lung Dose at Death, rad	Number of Animals		Survival Time (mean ± SD)		Lung Dose, rad (mean ± SD)		Lung Lesions: No. (%) of Metaplasias		Lung Lesions: No. (%) of Neoplasias	
	Cm	Am	Cm	Am	Cm	Am	Cm	Am	Cm	Am
<10	32	49	660 ± 205	696 ± 127	5.0 ± 2.4	5.7 ± 2.4	0	1 (2.0)	1 (3.1)	0
10-50	42	86	621 ± 180	728 ± 125	26 ± 11	25 ± 11	1 (2.4)	2 (2.3)	6 (14.3)	6 (7.0)
50-100	11	9	542 ± 117	709 ± 143	73 ± 15	70 ± 16	2 (18.2)	0	2 (18.2)	0
100-500	23	10	498 ± 81	629 ± 122	183 ± 91	210 ± 110	7 (30.4)	3 (30)	8 (34.8)	6 (60)
>500	23	15	68 ± 105	274 ± 258	901 ± 380	1500 ± 660	0	0	1 (4.3)	1 (6.7)

TABLE 2. Comparison of Bone Dose-Response in Rats Exposed by Inhalation to $^{241}AmO_2$ or $^{244}CmO_2$.

Bone Dose at Death, rad	Number of Animals		Survival Time (mean ± SD)		Bone Dose, rad (mean ± SD)		No. (%) of Bone Tumors	
	Cm	Am	Cm	Am	Cm	Am	Cm	Am
<10	74	153	615 ± 137	712 ± 128	2.4 ± 2.0	1.3 ± 1.6	3 (4.1)	1 (0.4)
10-50	21	12	602 ± 177	418 ± 289	27 ± 11	33 ± 11	1 (4.8)	0
50-100	19	6	389 ± 309	146 ± 10	69 ± 13	72 ± 11	3 (15.8)	0
100-500	38	0	368 ± 240	--	252 ± 114	--	4 (10.5)	--
>500	5	0	51 ± 78	--	655 ± 61	--	1 (20)	--

were found in rats exposed at 267 days than in those exposed at 70 days. We conclude that fractionation of inhaled $^{244}CmO_2$ increases the carcinogenic risk in both lung and bone, in contrast to the lack of effect from fractionating $^{239}PuO_2$ exposure, seen in earlier studies with inhaled $^{239}PuO_2$ (Annual Report, 1980).

When ^{244}Cm was given at 267 days of age, it appeared that insufficient time was available for full expression of the carcinogenic potential. No evidence of a shortened latent period was seen in this group.

Effect of Vitamin C on Pulmonary Carcinogenesis From Inhaled $^{239}PuO_2$

Vitamin C has been implicated as a nutritional agent that may be useful in preventing cancer. The objective of the study was to examine the effect of vitamin C (50% sodium ascorbate and 50% ascorbic acid) in drinking water on the development of lung tumors in rats previously exposed to high-fired $^{239}PuO_2$ aerosols.

Four groups of Fischer-344, young adult female rats were examined: 1) 28 otherwise untreated rats were exposed to $^{239}PuO_2$; 2) 32 rats were given 10 mg/ml vitamin C in drinking water; 3) 24 $^{239}PuO_2$-exposed rats were given 1 mg/ml vitamin C in drinking water; and 4) 25 $^{239}PuO_2$-exposed rats were given 10 mg/ml vitamin C in drinking water. Vitamin C therapy was continued for about 1 yr following inhalation of $^{239}PuO_2$. Initially, water consumption averaged about 30 ml per day, increasing to 40-45 ml per day after several months. No consistent

TABLE 3. Summary of Characteristics of Lung Deposition and Survival Following Single or Multiple Exposures of Rats to Inhaled $^{244}CmO_2$.

	Single Exposure		Multiple Exposures	Controls	
	70 Days of Age	267 Days of Age		Sham-Exposed	Shelf
Number of Rats	33	27	59	31	18
Initial Alveolar Deposition, μCi	92 ± 101	211 ± 308	101 ± 157	--	--
Dose to Lung at Death, rad	69 ± 64	152 ± 274	88 ± 143	--	--
Survival, Days $\overline{X}$ ± SD	439 ± 177	479 ± 151	505 ± 143	660 ± 93	663 ± 104

TABLE 4. Lung and Bone Lesions in Rats Exposed to Inhaled $^{244}CmO_2$.

	Percent of Animals with Lesions		
	Lung Lesions		
Group	Metaplasia	Tumors	Bone Tumors
Shelf Controls	6	0	0
Sham-Exposed Controls	6	3	0
Single Exposure (70 Days)	15	9	12
Single Exposure (267 Days)	22	4	0
Multiple Exposure	22	20	24

significant differences in water consumption were seen among the various groups.

Survival rate of Pu-exposed rats given 10 mg/ml vitamin C was less than that of all other groups, probably because of the higher dose (and subsequent deposition) of ^{239}Pu than in the other two exposed groups (Table 5). Survival was similar in all other groups.

The incidence of preneoplastic pulmonary metaplasias (squamous cell metaplasia and adenomatosis) was higher in Pu-exposed rats given vitamin C compared to rats given only ^{239}Pu. The reverse is true regarding the frequency of lung tumors (squamous cell carcinoma, adenocarcinoma and mixed squamous adenocarcinoma): more lung tumors were found in animals given only Pu (71%) than in animals given both Pu and vitamin C (41-45%). Tumor incidence was not related to the dose of vitamin C. The differences in lung tumor incidence were not statistically significant because of the radiation dose variability in groups and the small number of animals in each group.

Assessment of Cell-Mediated Immunity Following Inhalation of $^{239}PuO_2$

Immunological studies have been initiated in $^{239}PuO_2$-exposed rats because of the potential involvement of both humoral and cellular immunity in carcinogenesis from inhaled alpha-emitting radionuclides We examined T-lymphocyte distribution in spleen-cell preparations with ^3H-uridine uptake assay, and response of peripheral-blood and spleen lymphocytes to mitogen stimulation.

Fischer rats were divided into three groups of 30 rats each: 1) unexposed controls; 2) low-dose ^{239}Pu inhalation (IAD, 18.8 ± 7.2 nCi) and 3) high-dose ^{239}Pu inhalation (IAD, 163 ± 47 nCi). Mitogens tested were concanavalin-A (Con-A) and phytohemagglutinin (PHA), which stimulate T-cells; pokeweed mitogen (PWM), which stimulates mostly B-cells; and bacterial lipopolysaccharide (LPS), which is specific for stimulating B-cells.

Measurements were performed at 30 days and 105 days after exposure. (A third group will be examined at 240 days after exposure). At 30 days, no significant effects were seen in mitogen stimulation or distribution of T-cells in the spleen. Spleen and whole-body weights were not different in exposed rats and controls. At 105 days, there was a statistically significant reduction in the response of spleen cells to LPS and PWM in both the low- and high-dose exposure groups. Responsiveness of Con-A and PHA were also reduced (although not significantly) at

TABLE 5. Role of Vitamin C Administered in Drinking Water of Rats with $^{239}PuO_2$-Induced Pulmonary Carcinogenesis.

	Pu-Exposed			
	No Therapy	Vitamin C, 1 mg/ml	Vitamin C, 10 mg/ml	Vitamin C, 10 mg/ml
No. of Rats	28	24	25	32
Survival, Days ($\bar{X}$ ± SD)	560 ± 100	510 ± 130	430 ± 100	570 ± 130
Initial Alveolar Deposition, μCi ($\bar{X}$ ± SD)	140 ± 120	140 ± 130	240 ± 260	--
Lung Dose at Death, rad ($\bar{X}$ ± SD)	2500 ± 2200	2400 ± 2300	3500 ± 3400	--
Lung Metaphasias, %	14	23	45	7
Lung Tumors, %	71	41	45	4

these times. The uptake of ^{3}H-uridine by spleen cells was decreased in the high-dose group, suggesting a reduction in spleen T-cells.

The response of peripheral-blood lymphocytes to Con-A and PHA was not significantly different, in exposed rats, at 105 days after exposure from that of controls; however, the PWM response was significantly increased over that of controls at both low and high ^{239}Pu levels. These preliminary data suggest an alteration in humoral immune responsiveness that could lead to a reduced antibody response to antigen challenge in Pu-exposed rats. No lymphopenia was observed in any Pu-exposed rats, suggesting that lymphocytes were actively recruited from the spleen to replace those irradiated in the alveolar capillaries.

Radiosensitivity of Macrophage Bactericidal Function

Studies last year (Annual Report, 1980) indicated that thoracic gamma or neutron irradiation significantly inhibited macrophage-related clearance of inhaled $^{239}PuO_2$. Alveolar macrophages are involved in the phagocytosis and killing of inhaled bacteria, and, possibly, tumor cells. Therefore, damage to macrophages may result in altered bactericidal capability and increased susceptibility to lung infection and tumor formation. Rabbit pulmonary alveolar macrophages were cultured with Staphylococcus aureus following ^{60}Co gamma-irradiation with 1000, 3000, 5000 or 10,000 rad. The percent of bacteria remaining alive was determined by counting the number of bacteria in macrophage culture vs. the number of bacteria in culture without macrophages. There appeared to be a linear decrease in the ability of macrophages to kill bacteria with increasing dose; only at 10,000 rad was the depression in bactericidal ability statistically significant ($P = <0.05$; Figure 1).

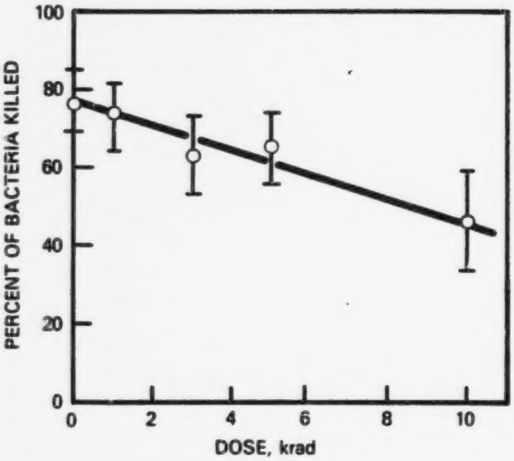

FIGURE 1. Bactericidal Action of Rabbit Alveolar Macrophages Following Irradiation with ^{60}Co Gamma-Rays. (Values are means ± SD [n, 8-11].)

• Low Level $^{239}PuO_2$ Life-Span Studies

Principal Investigator: C. L. Sanders

Other Investigators: J. A. Mahaffey and K. E. McDonald

The purpose of this project is to determine the dose-response curve for lung-tumor incidence in rats following inhalation of $^{239}PuO_2$ at levels producing a lifespan radiation dose of 2 to 100 rad.

In preliminary studies, rats were intratracheally instilled with, or inhaled ^{169}Yb-$^{239}PuO_2$ or ^{169}Yb-Yb_2O_3 particles. Whole-body, excreta and tissue levels of ^{169}Yb were determined by external gamma counting; ^{239}Pu levels were determined radiochemically by scintillation counting. It was found that ^{169}Yb dissociated from $^{239}PuO_2$ and was cleared more rapidly from the lung and the whole body. Despite this fact, however, whole-body counts performed at 7 days after exposure to ^{169}Yb-$^{239}PuO_2$ gave a reproducible measure of the ^{239}Pu lung burden.

Initial studies with $^{237,239}PuO_2$ particles for the purpose of estimating initial lung deposition by external whole-body counting demonstrated the role of dose in $^{239}PuO_2$ clearance (Annual Report, 1980). However, the cost and difficulty of obtaining sufficient amounts of ^{237}Pu has forced us to seek alternatives. Previous work done at the Inhalation Toxicology Research Institute (ITRI) with ^{169}Yb as a gamma tag for Pu, indicated that it might be well suited to our needs; it has a short half-life (32 days) and a mean gamma energy of 100 KeV, suitable for external counting.

A series of short-term dosimetric studies were performed with Fischer rats to determine the accuracy of estimating initial alveolar deposition of ^{239}Pu using ^{169}Yb-tagged $^{239}PuO_2$ particles. Exposures to the lung were by intratracheal instillation or by inhalation of freshly prepared ^{169}Yb-$^{239}PuO_2$ or ^{169}Yb-Yb_2O_3. The labeled oxides were prepared by adding ^{169}Yb to nitric acid solutions of Yb_2O_3 or $^{239}PuO_2$. The plutonium solution was reduced with hydrazine and ascorbic acid to Pu(III). Oxalic acid was added to both the ytterbium and plutonium solutions to precipitate the insoluble oxalates, which were fired at 750°C for 2 hours in air to form the labeled oxides. These were ground and suspended in triple-distilled H_2O for aerosolization. The average AMAD (N = 8) of the aerosolized particles for all exposures was 1.578 ± 0.106; GSD, 2.15 ± 0.324; CMD, 0.099 ± 0.056.

Whole-body gamma counts of exposed animals were performed in a lead-shielded cave containing two banks of thirteen 2-inch NaI crystals. At necropsy, individual tissues were removed and gamma counted for ^{169}Yb. Subsequent to gamma counting, the tissues were ashed and ^{239}Pu determined by liquid scintillation counting.

In the first study, 80 rats were intratracheally instilled wih either 2.0 nCi ^{169}Yb as ^{169}Yb-Yb_2O_3 particles, or 2.0 nCi ^{169}Yb plus 4.0 nCi ^{239}Pu as ^{169}Yb-$^{239}PuO_2$ particles. The lung clearance curve, as determined by ^{169}Yb gamma counting, was similar for ^{169}Yb-Yb_2O_3 particles and for ^{169}Yb-$^{239}PuO_2$ particles. The lung clearance of ^{169}Yb was, however, considerably faster than that of ^{239}Pu (as determined by scintillation counting; Figure 1). The greater solubility of ^{169}Yb, whether as Yb_2O_3 alone or as Yb_2O_3 mixed with $^{239}PuO_2$, was apparent from the greater loss of the radionuclide from the lung and from the larger amount excreted in urine and feces. The estimated lung clearance at 30 days after exposure was 29% for ^{239}Pu and 60-63% for ^{169}Yb (either as ^{169}Yb-Yb_2O_3 or as ^{169}Yb-$^{239}PuO_2$) when intratracheally instilled.

In the second study, 80 rats inhaled an aerosol of ^{169}Yb-$^{239}PuO_2$ particles and were killed at intervals from 3 to 58 days after exposure. The results were similar to those seen with the intratracheally instilled radionuclides, although inhaled ^{169}Yb and ^{239}Pu were cleared from the lung somewhat faster than the instilled radionuclides (Figure 1). The lung clearance at 30 days after exposure was 45% for inhaled ^{239}Pu as ^{169}Yb-$^{239}PuO_2$, 50% for inhaled ^{239}Pu as $^{239}PuO_2$, and 67% for inhaled ^{169}Yb as ^{169}Yb-$^{239}PuO_2$. In both instillation and inhalation studies, ^{169}Yb was preferentially dissolved from ^{169}Yb-$^{239}PuO_2$, causing a more rapid loss of ^{169}Yb than of ^{239}Pu from the lung. Most of the ^{169}Yb was excreted rapidly in urine and feces--twice as much of the initial

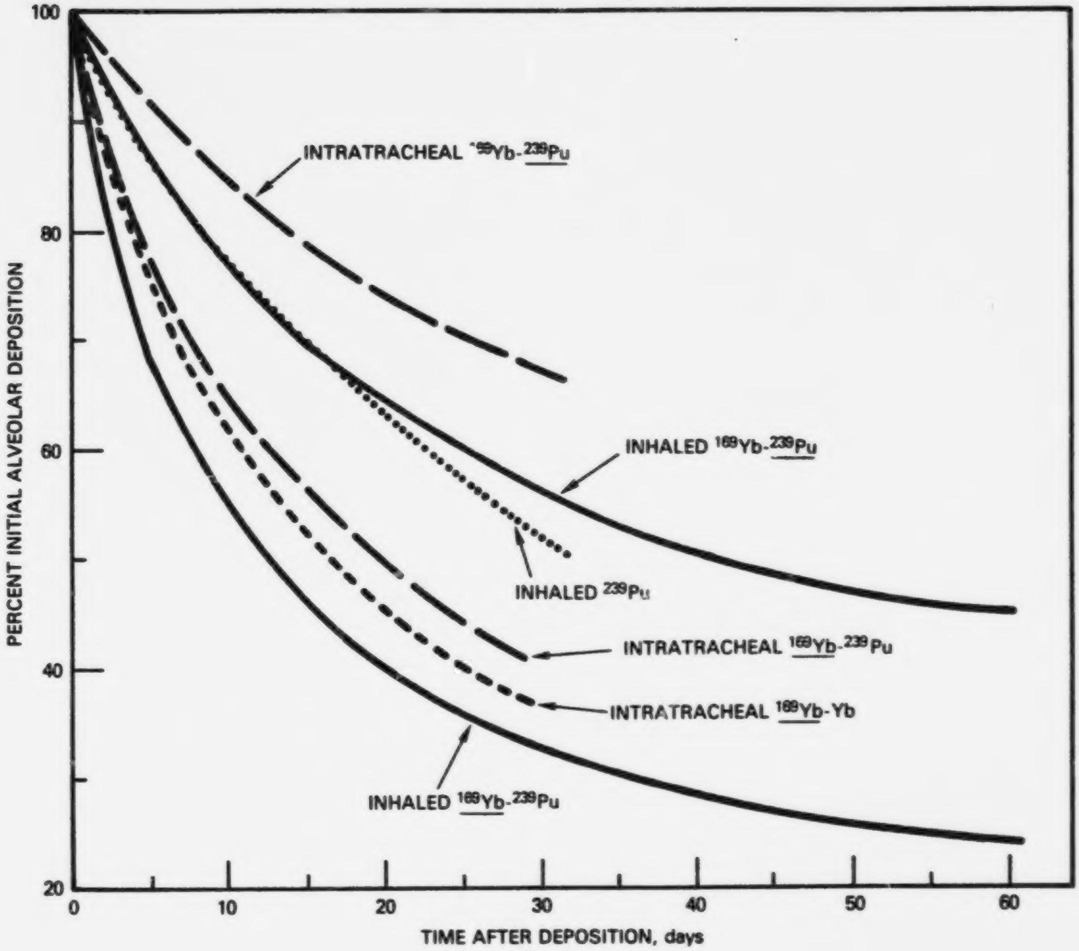

FIGURE 1. Lung Clearance of Intratracheally Instilled or Inhaled ^{169}Yb or ^{239}Pu as ^{169}Yb-Yb_2O_3 or ^{169}Yb-$^{239}PuO_2$ Particles. Radionuclide measured for each curve is underlined.

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alveolar deposition of ^{169}Yb as that of ^{239}Pu.

In the third study, 105 rats were exposed to ^{169}Yb-$^{239}PuO_2$ particles by inhalation. Whole-body counts of ^{169}Yb were made at 1, 3 and 7 days after exposure. Groups of 35 animals were killed at 1, 3 and 7 days and their lungs analyzed for ^{239}Pu by scintillation counting. A regression analysis was performed for each time on estimated ^{239}Pu in lung (based on whole-body ^{169}Yb counting) and actual ^{239}Pu in lung (as determined by scintillation counting [Table 1]). This analysis indicates a poor relationship between estimated and actual ^{239}Pu lung burdens at 1 and 3 days after exposure. However, a significant correlation ($R^2 = 0.85$) was obtained at 7 days after exposure (Table 1, Figure 2). Despite the fact that ^{169}Yb was shown in earlier experiments to leave the lung faster than ^{239}Pu, estimates of ^{239}Pu retention based on ^{169}Yb counting are high. At early sacrifice times this is, no doubt, due to ^{169}Yb retention in the GI tract and other extra pulmonary tissues, but the overestimate at 7 days is somewhat surprising.

A fourth short-term study is in progress to extend these observations.

TABLE 1. Comparison of Measured ^{239}Pu Lung Burden with Estimated ^{239}Pu Lung Burden (from ^{169}Yb whole-Body Counting). Values are mean ± SD (N = 35).*

	Plutonium in Lung, nCi			
Time After Exposure, days	Estimated from Whole-Body ^{169}Yb Count (X)	Measured (Y)	Regression Equation	R^2
1	16 ± 7.8	4.4 ± 1.7	$Y = 1.8 + 0.16X$	53
3	7.7 ± 2.8	3.7 ± 1.8	$Y = -0.026 + 0.49X$	59
7	5.5 ± 2.3	3.8 ± 1.9	$Y = -0.25 + 0.74X$	85

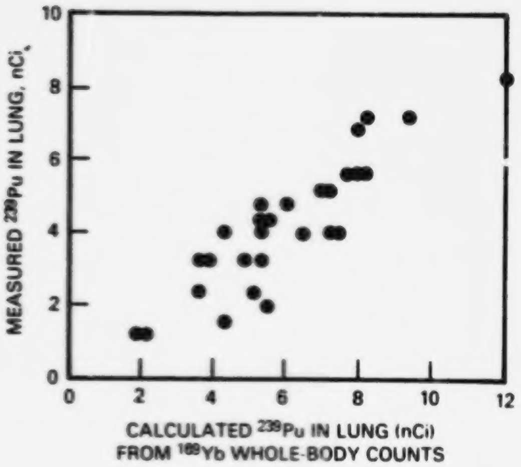

FIGURE 2. Scatterplot of Measured ^{239}Pu in Lung (Scintillation Counting) and Estimated ^{239}Pu in Lung (from Whole-Body Counting of ^{169}Yb) at 7 Days After Exposure to ^{169}Yb-^{239}PuO$_2$ Aerosol.

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*There is a poor correlation on days 1 and 3 of the estimated Pu present, based upon whole-body gamma counting and that which is actually present as determined by liquid scintillatin. This is due to variable early gastrointestinal depositions of ^{169}Yb due to licking or external contamination and early nasal and tracheal clearance. The transit time through the GI tract of the rat is about 3 days.

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• Toxicology of Plutonium-Sodium

Principal Investigator: P. L. Hackett

Other Investigators: M. D. Allen, J. K. Briant, A. C. Case, D. D. Mahlum, L. G. Morgan, G. J. Powers, and J. L. Ryan

Technical Assistance: E. F. Blanton, A. J. Clary, R. L. Music, E. J. Rossignol, and S. A. Watson

Scenarios for liquid-metal fast breeder reactor (LMFBR) accidents predict the loss of sodium coolant, with subsequent core melt-down and release of mixed sodium-fuel aerosols [$Na-(PuU)O_2$] into the environment. Studies in other laboratories demonstrated that mixed aerosols of Na_2O-PuO_2 were more readily transported from the lung than PuO_2 aerosols. We therefore devised a continuous aerosol-generating system for animal exposures in which laser-generated fuel aerosols were swept through sodium vapor to form sodium-fuel aerosols. These fuel and sodium-fuel aerosols were compared with regard to their physicochemical properties and their biological behavior following inhalation studies in rats and dogs.

Previous studies concerning physicochemical characterization of laser-generated fuel aerosols revealed that they were enriched in uranium and oxygen relative to the fuel. The fuel aerosols consisted of branched-chain aggregates of very small particles with an activity median aerodynamic diameter (AMAD) of <1 µm. Sodium-fuel aerosols were spherical, with AMADs proportional to the sodium-fuel ratio and larger than those of the fuel aerosols.

In vitro solubility of the mixed aerosols was greater than that of fuel aerosols. Enhanced solubility, in vivo, of the mixed aerosols was indicated by the appearance of larger fractions (compared to those of fuel aerosols) of the initial lung burden in blood, liver and skeleton of rats or dogs following inhalation exposure.

Chemical studies designed to explore mechanisms for the enhanced solubility of the fuel-sodium aerosols revealed that uranium was rapidly converted to a soluble uranyl carbonate complex-ion species in the presence of air and moisture. A small quantity of atomically dispersed plutonium results from destruction of the fluorite-type lattice when uranium dissolves. Results from investigations of plutonium oxidation states in sodium-PuO_2 aerosols are consistent with the chemical reactions $Na + 3PuO_2 \rightarrow Pu_2O_3 + NaPuO_3$.

It was apparent from chemical studies that release of a more soluble form of Pu into the environment could significantly alter deposition and distribution of Pu in pregnant individuals. A previous study (Annual Report, 1979) involving inhalation exposure of pregnant rats late in gestation (19 days) indicated that fetoplacental depositions of ^{239}Pu from sodium-fuel aerosols exceeded that of the fuel aerosols. We have continued these studies to determine fetal deposition patterns following maternal exposure to smaller sodium-fuel particles (2.4 versus 3.5 µm) at earlier stages of gestation.

In the current study, we exposed Wistar rats at 15 or 19 days gestation (dg) for 1 hr, nose-only, to a LMFBR-fuel aerosol or a sodium-fuel aerosol (Table 1). Groups of 5 or 6 rats were killed immediately after exposure, at 1 day, or at 5 days for ^{239}Pu analysis of maternal and fetal tissues. Results from these analyses were compared following normalization of aerosol values to 40 nCi/L.

TABLE 1. Characteristics of LMFBR-Fuel and Sodium-Fuel Aerosols.

Characteristic	Fuel	Sodium-Fuel
Sodium-Fuel Ratio	0:1	40:1
^{239}Pu Concentration, nCi/L	210	40
AMAD/GSD[a]	0.66/1.50	2.40/1.41

[a] Activity median aerodynamic/geometric standard deviation.

Initial lung burdens for maternal rats inhaling fuel aerosols appeared lower than

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those of rats exposed to the sodium-fuel but were not significantly different (Table 2). Five days after exposure, lung values for rats exposed to sodium-fuel were significantly lower than initial lung burdens. By comparison, lung clearance of fuel aerosols was somewhat reduced. Maternal blood values for ^{239}Pu in sodium-fuel-exposed rats were markedly higher than those of the fuel-exposed rats during the entire experimental interval. These differences were reflected in the ^{239}Pu values for components of the fetoplacental units (FPU).

TABLE 2. Lung and Blood Values (Mean ± SE) in Pregnant Rats Following Exposure to Fuel or Sodium-Fuel Aerosols (Normalized to 40 nCi/L).

Tissue	Days After Exposure	Aerosol: Fuel	Aerosol: Sodium-Fuel
Lungs, nCi	0	45 ± 3(a)	62 ± 8(a)
	1	47 ± 4(a)	57 ± 6(a)
	5	38 ± 3(a,b)	29 ± 3(b)
Blood, pCi/ml	0	0.34 ± 0.18(a)	17.1 ± 2.9(c)
	1	0.15 ± 0.04(a)	5.8 ± 0.9(b)
	5	0.16 ± 0.06(a)	5.8 ± 0.8(b)

(a-c) Values with common letters for a given tissue are not significantly different (P < 0.05).

Plutonium deposition within the FPU was highest in membranes, next highest in placenta, and lowest in fetuses (Table 3). Plutonium-239 accumulated more readily in the larger, more-developed structures of the 19-dg than in the 15-dg FPU. Values for the 20-dg FPUs were not significantly different, although the 15-dg sodium-fuel-exposed FPU appeared to acquire more ^{239}Pu over the 5-day exposure than did the 19-dg FPU during a 1-day exposure. These results demonstrate that fetal accumulation of ^{239}Pu following exposure to the more-soluble sodium-fuel aerosols is 60 to 80 times greater than fetal deposition following fuel-aerosol exposure. The data also imply that exposure to these aerosols earlier in fetal development, or even prior to implantation, may enhance fetal accumulation of ^{239}Pu during the longer period of intrauterine exposure.

The results of this project clearly demonstrate that in order to fully evaluate the potential effects of Pu released to the environment from reactor accidents, it is important to understand the biological behavior of complex mixtures of the sodium actinides.

TABLE 3. Distribution of ^{239}Pu Within the Fetoplacental Unit of Rats that Inhaled LMFBR-Fuel or Sodium-Fuel Aerosols (pCi, Mean ± SD).

Tissue	Days After Exposure	Fuel(a) 15 dg(b)	Fuel(a) 19 dg(b)	Sodium-Fuel(a) 15 dg(b)	Sodium-Fuel(a) 19 dg(b)
Placenta	0	0.04 ± 0.01(c,d)	0.01 ± 0.002(c)	2.2 ± 0.3(e)	6.1 ± 1.6(e,f)
	1	0.02 ± 0.003(c)	0.05 ± 0.005(d)	3.1 ± 0.4(e)	16 ± 2.5(f,g)
	5	0.14 ± 0.04(d)		17 ± 1.6(g)	
Membranes	0	0.02 ± 0.005(c)	0.02 ± 0.002(c)	0.5 ± 0.1(e)	3.6 ± 1.5(c,d,e)
	1	0.02 ± 0.003(c)	0.11 ± 0.02(d)	5.5 ± 0.9(f)	34 ± 4.7(g)
	5	0.46 ± 0.16(c,d,e)		60 ± 8.4(g)	
Fetus	0	0.05 ± 0.02(c,d)	0.04 ± 0.01(c,d)	0.3 ± 0.06(e,f)	0.7 ± 0.2(f)
	1	0.01 ± 0.002(c)	0.03 ± 0.006(c,d)	0.2 ± 0.02(e)	1.9 ± 1.9(g)
	5	0.03 ± 0.003(d)		2.5 ± 0.5(g)	

(a) Aerosol activities normalized to 40 nCi/L.

(b) Days of gestation at exposure

(c-g) Values with common letters for a given tissue are not significantly different (P <0.05).

• Cigarette Smoke and Plutonium

Principal Investigator: R. E. Filipy

Other Investigators: W. J. Bair, J. L. Pappin, and D. L. Stevens

Technical Assistance: J. C. Chapman, J. S. Cunningham, S. G. Irby, and C. R. Petty

The major objective of this project is to obtain experimental data that are directly applicable to resolving the question of whether cigarette smokers are at greater risk than nonsmokers to potential health effects of inhaled plutonium. Because cigarette smokers constitute a large fraction of the population, a synergistic effect of plutonium and cigarette smoke might influence estimates of the health risk for plutonium and other transuranics released to the environment.

Progress was made on two fronts during the past year. The autoradiographic technique developed for detection of plutonium on the interior surface of pulmonary airways (Annual Report, 1978) has been adapted to routine use in examining tracheas and bronchi of rats. Also, dogs exposed to cigarette smoke for over a year after inhalation of plutonium were killed and necropsied.

Distribution of $^{239}PuO_2$ in Pulmonary Airways of Rats

Briefly, the autoradiographic technique involves removal of the mucosal layer from a pulmonary airway and affixing it to a glass microscope slide. This sheet of epithelial tissue is then coated with liquid photographic emulsion, exposed, and developed to reveal plutonium particles on what was the inside surface of the airway. A grid system is superimposed on the microscope slide and the number of alpha tracks and stars in each square of the grid is determined via a microscope. Thus, very small plutonium particles (tracks) and larger particles with multiple radioactive disintegrations (stars) are characterized with respect to location within pulmonary airways at the time of the animal's death.

Routine application of the autoradiographic technique was begun with tracheas and primary bronchi from rats which had been exposed to cigarette smoke for 7 mo, followed by exposure to $^{239}PuO_2$ aerosols and 6 wk of subsequent smoke exposure before sacrifice. Particle counts in tracheas of nine of these rats have been completed. The number of stars or individual tracks was relatively constant among animals, regardless of the lung burden: 65 ± 14 (mean ± SD) stars (range = 36 to 89 stars) in the tracheas of all nine rats. Both stars and tracks appear to be quite uniformly distributed on the surface of the tracheas, although there are areas of heavier concentration in some rats.

These data, however, do not provide information as to whether the plutonium particles were lodged in the trachea during or shortly after exposure or whether the particles were progressing up the mucociliary escalator at the time of death. Quantitative data from rats killed sooner after plutonium inhalation, and from plutonium-exposed rats which received no cigarette smoke, may allow some conclusions in that respect. Collection of those data is still in progress.

Clearance of $^{239}PuO_2$ from the Lungs of Dogs

Two groups of dogs were exposed to a plutonium aerosol, one group after 6 mo of exposure to cigarette smoke, the other with no smoke exposure. Each group consisted of four dogs which had been previously exposed to smoke (or sham-exposed) for nearly 10 yr as part of another project, and two young-adult dogs with no previous exposure. (For ease in referring to these groups, we will call them "young" or "old" "smokers" or "nonsmokers.") Cigarette-smoke exposure or sham exposure was resumed 20 days after inhalation of plutonium and continued for approximately 60 wk, until the dogs were killed. Weekly, in vivo, whole-body counts were made during that period. Feces were collected for radioanalyses for the first 250 days after Pu exposure.

The primary purpose of this experiment with dogs was to test smoke delivery and plutonium detection methods, therefore small numbers of dogs were used. When the data are examined retrospectively, however, interesting trends appear even though group differences are not always statistically significant.

Results of whole-body counts are presented in Figure 1. The greatest differences in pulmonary clearance rates were between young smokers and young nonsmokers, and between young and old nonsmokers. Differences between old smokers and old nonsmokers, and between old and young smokers, were less pronounced.

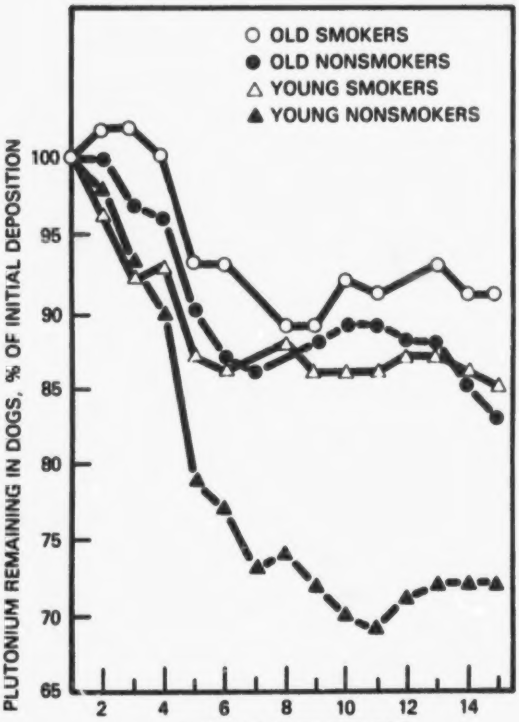

FIGURE 1. Results of In Vivo Detection (Whole-Body Counts) of Plutonium in Smoke-Exposed and Sham-Exposed Dogs.

TABLE 1. Percent of Initial Alveolar Burden of $^{239}PuO_2$ Excreted in Feces and Remaining in Selected Tissues at Death of Smoke-Exposed and Sham-Exposed Dogs.

Dog No.	IAB[a], nCi	Total Fecal Excretion (Days 4-251), % of IAB	Tracheobronchial Lymph Nodes, % of IAB	In Vitro Lung Count[b], % of IAB
Nonsmokers				
Old				
602[c]	323	21	10	58
604	351	11	2	54
584	668	6	29	44
628	398	9	6	41
Young				
1733	1450	3	6	33
1668	1329	5	34	35
Smokers				
Old				
554	515	6	2	55
569	296	10	6	61
594	618	5	7	53
599	537	4	2	55
Young				
1722	1140	4	13	52
1700	890	4	15	40

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[a] Initial alveolar burden (IAB) based on the first four whole-body counts (days 6-18 after plutonium exposure).

[b] Individual lobes of excised fixed lungs were placed in the whole-body counter.

[c] Dog 602 died ~100 days before the others were sacrificed, therefore lung and lymph node data were omitted from statistical computations.

It became obvious that whole-body counts became less reliable with time as a measure of the lung burden of plutonium, even though they were useful for comparative purposes. In individual dogs, the counts generally decreased between plutonium inhalation and 200 days thereafter. In five of eight old dogs and one of four young dogs, the counts increased after that time; for dog 594 (Table 1), the final counts indicated 112% of the initial body burden. Because no additional plutonium was available after the initial exposure, the increase in counts must be attributed to redistribution of plutonium in the lung, with resulting increased detectability. The phenomenon, in dogs, of plutonium migration toward the pleura was previously demonstrated in this laboratory (Annual Report, 1980). With the young nonsmokers, pulmonary clearance apparently occurred rapidly enough so that whole-body counts were less affected by the migration of plutonium.

The results of lung counts made on fixed, excised lungs, after necropsy, are probably more reliable than whole-body counts because they are relatively unaffected by redistribution and because the tracheobronchial lymph nodes were removed before counting. Those data (Table 1) indicate differences in the amount of plutonium remaining in lungs of old and young dogs, and in smokers and nonsmokers. The smoke-exposed dogs retained significantly (P <

0.06) more plutonium in their lungs than did the nonsmokers. Combining old dogs of both groups and young dogs of both groups, old dogs retained significantly ($P < 0.03$) more plutonium in their lungs than did young dogs.

The cumulative fecal excretion of plutonium is illustrated in Figure 2. There was virtually no difference in the rate of fecal excretion between young smokers and young nonsmokers. Old dogs excreted plutonium at a significantly greater rate ($P < 0.04$) than young dogs. The fecal excretion rate was significantly greater ($P < 0.09$) in old nonsmokers than in old smokers, and the difference between all young dogs and the old smokers was significant ($P < 0.06$).

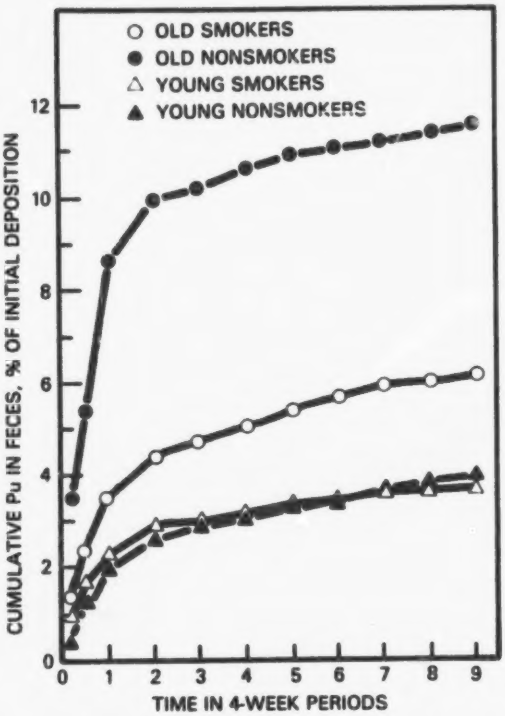

FIGURE 2. Cumulative Fecal Excretion of Plutonium by Smoke-Exposed and Sham-Exposed Dogs.

It is difficult to know why total fecal excretion of plutonium (Table 1) was less in young dogs than in old, even though pulmonary clearance seems to be considerably more effective in the young dogs, as indicated by in vitro lung counts. A possible explanation for reduced fecal excretion in young dogs is that their lymphatic clearance was more effective than that of the old dogs: three of four young dogs and one old dog (584) had greater-than-average plutonium concentrations in the tracheobronchial lymph nodes (Table 1). These four dogs excreted less than the average amount of plutonium.

A few conclusions, some of which are quite speculative, may be drawn from the data presented in Figures 1 and 2 and Table 1. In dogs, cigarette smoke exposure appears to decrease the pulmonary clearance rate of inhaled plutonium. The effect seems greater in young dogs than in old dogs; the latter have a slower clearance rate than young dogs whether or not they have been exposed to cigarette smoke.

All lungs of these dogs were fixed in an inflated state and are currently in storage. The airways of these lungs will either be subjected to the autoradiographic technique, as discussed above, or with cellulose nitrate track etch film. To provide another set of pulmonary retention data, lung parenchyma not used in these techniques will be radiochemically analyzed for plutonium.

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• Toxicity of Sodium-Lithium

Principal Investigator: R. H. Busch

Other Investigators: J. K. Briant, T. M. Graham, K. E. Lauhala, K. E. McDonald, J. E. Morris, and E. J. Rossignol

Technical Assistance: S. D. Harris

Rats were exposed to inhaled sodium carbonate aerosols (2600 μg/L) to detect possible effects on immunologic responsiveness. Immunologic testing revealed reduced response of spleen cells to mitogens and a reduced proportion of T-lymphocytes in spleen-cell preparations during the period 3-4 days after exposure.

The objective of this project is to determine possible toxic effects of aerosolized reaction products of sodium and lithium that might be released from accidental breaks in reactor coolant systems employing these metals as heat transfer agents. Such information is needed to determine exposure levels which would constitute a potential hazard to exposed populations, and to determine toxic effects, so that protective measures could be devised.

In earlier attempts to establish an LC_{50} for sodium carbonate, we performed a series of whole-body inhalation exposures of rats, mice, and guinea pigs to varying concentrations (from 1500 μg/L to 3600 μg/L) of the aerosol. The animals (adult male Wistar rats, adult male Sprague-Dawley rats, adult male Swiss-Webster mice and adult male Hartley albino guinea pigs) were exposed in a chamber previously described (Annual Report for 1978). Relative humidity, temperature and oxygen concentration were monitored and controlled during each exposure. For each exposure we measured average chamber aerosol concentration in μg/L, % Na_2CO_3, % NaOH, % $NaHCO_3$, and activity median aerodynamic diameter/geometric standard deviation (AMAD/GSD) of particles. Results of these studies have been reported (Annual Reports for 1976 to 1980).

These studies have now been expanded to include measurement of possible alterations in immunologic responsiveness. To accomplish this, 48 rats were exposed to a single aerosol concentration (2600 μg/L).

Cellular immunity in rats killed at 3-4 days and at 12-13 days after sodium inhalation was assessed, using mitogen-induced lymphocyte activation assays and T-cell distribution assays. Lymphocytes from spleen and peripheral blood were tested with the mitogens concanavalin-A (Con-A), phytohemagglutinin (PHA), pokeweed mitogen (PWM) and lipopolysaccharide (LPS). Spleen-cell population was assayed for T-cell distribution, based on uptake of ^3H-uridine.

The response to PHA of spleen lymphocytes from exposed rats killed 3-4 days after exposure was significantly reduced ($P < 0.05$) when compared to that of control animals. Although stimulation of spleen cells with Con-A, PWM and LPS was also less for treated than for control rats, the difference was not statistically significant. Differences between peripheral-blood lymphocyte responses were also statistically insignificant. The response of spleen and peripheral-blood lymphocytes from exposed rats killed 12-13 days after exposure was not statistically different from that of controls.

The proportion of T-lymphocytes in spleen-cell preparations from rats killed 3-4 days after exposure was reduced compared to controls; this was not the case in rats killed 12-13 days after exposure.

These results suggest a transitory immunologic repression. This repression may contribute to the bacteremia observed during the 1- to 14-day postexposure period.

Lithium studies have thus far been restricted to generating and controlling aerosols of lithium combustion products. Technical problems have been encountered in producing aerosols of sufficiently high concentration to cause effects. Modification of the generating equipment will be necessary to attain desired aerosol concentrations of predetermined particle size and chemical state. Subacute-level lithium exposures are expected to commence in 1982.

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• Inhalation Hazards to Uranium Miners

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Technical Assistance: C. R. Petty, P. Pottmeyer, and W. Skinner

This project is investigating levels of uranium mine air contaminants, using both large and small experimental animals to model human respiratory system disease. Lung cancer and deaths by degenerative lung disease have reached epidemic proportions among uranium miners, but the cause-effect relationships for these diseases are based on inadequate epidemiological data. This project identifies agents or combinations of agents (both chemical and radiological) and their exposure levels that produce respiratory tract lesions, including respiratory epithelial carcinoma, pneumoconiosis and emphysema.

Small-Animal Studies

Approximately 1100 male specific-pathogen-free Wistar rats are currently on study; the 4000 and 5000 Series experiments (Tables 1 and 2) are designed to clarify the roles of unattached RaA daughters, and the degree of radon daughter disequilibrium, on the development of respiratory system disease. The 6000 and 7000 Series experiments (Table 3) are designed to develop the relationships between response and exposure to radon daughters (at two rates of exposure) and carnotite uranium ore dust. The exposures of 6000 and 7000 Series animals are currently in progress; the exposures of 4000 and 5000 Series animals are completed; a few of the animals are still living.

TABLE 1. Radon-Daughter Unattachment Fraction Study in Rats (4000 Series experiments).

Exposure Regimen[a]	Total Exposure, WLM[b]
500 WL Radon Daughters Low RaA Unattachment (~2%) ~15 mg/m^3 Uranium Ore Dust	5120
500 WL Radon Daughters Intermediate RaA Unattachment (~10%) ~0.7 mg/m^3 Uranium Ore Dust	5120
500 WL Radon Daughters High RaA Unattachment (~24%) ~0.4 mg/m^3 Uranium Ore Dust	5120
Controls	

[a] 32 animals in each group, exposed 90 hr/wk

[b] Working Level (WL) is defined as any combination of the short-lived radon daughters in 1 liter of air that will result in the ultimate emission of 1.3 x 10^5 MeV of potential α-energy. Working Level Month (WLM) is an exposure equivalent to 170 hr at a 1-WL concentration.

TABLE 2. Radon-Daughter Disequilibrium Study in Rats (5000 Series experiments).

Exposure Regimen[a] Daughter Equilibrium Ratios				Total Exposure, WLM[b]
Rn	RaA	RaB	RaC-C′	
1	0.9	0.4	0.2	5120
1	0.5	0.07	0.01	5120
Controls				

[a] 32 animals in each group, exposed 90 hr/wk at 500 WL and 15 mg/m^3 uranium ore dust

[b] Working Level (WL) is defined as any combination of the short-lived radon daughters in 1 liter of air that will result in the ultimate emission of 1.3 x 10^5 MeV of potential α-energy. Working Level Month (WLM) is an exposure equivalent to 170 hr at a 1-WL concentration.

We have concluded that the most significant lesions related to radon daughter and carnotite ore-dust exposures in the 4000 Series experiments are malignant tumors of the respiratory tract. Histopathologic data for these tumors in the low and high RaA-unattachment groups are shown in Table 4. The tissues of animals of the intermediate RaA unattachment group, and for the 5000 Series experiments, are currently undergoing histopathologic examination.

The data in Table 4 suggest that a low RaA unattachment fraction (~2%) or high concentration of dust (~15 mg/m^3) is associated with a higher risk of malignant lung tumor development than a high RaA unattachment fraction (~24%) or low dust concentration (~0.4 mg/m^3). However, this interpretation must be delayed until a

TABLE 3. Exposure-Response Relationship Study for Radon-Daughter Carcinogenesis in Rats (6000 Series experiments).

Number of Animals[a]	Exposure Regimen[b,c]	Total Exposure, WLM[d]
32	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	10,240
32	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	5120
32	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	2560
32	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	1280
64	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	640
128	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	320
32	Controls	

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[a] Number of animals is sufficient to detect the predicted incidence of lung tumors at the 0.05 to 0.1 level of significance, assuming linearity of response between 0 and 9200 WLM (see footnote d), and 0.13% spontaneous incidence.

[b] Exposure rate, 90 hr/wk; planned periodic sacrifice

[c] Study will be repeated @ 100 WL rate (without periodic sacrifice) to augment previous limited exposure-rate data (7000 series experiments).

[d] Working Level (WL) is defined as any combination of the short-lived radon daughters in 1 liter of air that will result in the ultimate emission of 1.3×10^5 MeV of potential α-energy. Working Level Month (WLM) is an exposure equivalent to 170 hr at a 1-WL concentration. Previous exposure at 900 WL for 84 hr/wk to 9200 WLM produced a 60% incidence of carcinoma.

TABLE 4. Current Summary of Malignant Respiratory Tract Tumors (4000 Series experiments).

Malignant Respiratory Tract Tumors	RaA Unattachment Groups			
	Low	Intermediate	High	Controls
Lung				
Epidermoid Carcinoma	9	--	2	0
Adenocarcinoma	9	--	5	1
Malignant Mesothelioma	1	--	0	0
Total Number of Rats	16[a]	--	7	1
Nose				
Nasal Carcinoma	1	--	2	0
Total Number of Rats with Respiratory Tumors	17	--	8[b]	1

---

[a] Two rats had both epidermoid carcinomas and adenocarcinomas, and one rat had two types of adenocarcinomas.

[b] One rat had both a lung tumor and a nasal tumor.

competing risk analysis is performed on the data. (The mean survival times of the exposed animals were significantly [$P < 0.001$] reduced from those of controls and was shortest in the high RaA unattachment group of animals.) Although of doubtful significance, the high RaA unattachment exposure was associated with a higher incidence of nasal carcinoma.

Large-Animal Studies

Thirty-five beagle dogs are currently on study to determine the pathogenic role of inhalation exposure to carnotite uranium ore dust. We are particularly interested in clarifying the role of the ore dust in the production of the massive pulmonary fibrosis observed in an earlier study, in which beagle dogs were exposed to radon daughters and mixtures of uranium ore dust and cigarette smoke. The present study (chronic, head-only exposures) began when the dogs were about 2½ years old. Along with routine physical examinations and periodic hematologic and clinical chemistry measurements, histopathologic, morphometric and pulmonary function evaluations were conducted on these dogs. The histopathologic and morphometric data from sacrificed animals are shown in Table 5.

The most consistent pulmonary-function change attributed to carnotite uranium-ore-dust exposure (at ~15 mg/m^3, for 4 hr/day, 5 days/wk) is an increased slope of the single-breath N_2 washout curve, suggesting an uneven distribution of ventilation. This change was observed in dogs exposed for less than 1 year and continued through 4 yr of exposure. Measurements of pulmonary resistance, made after 27, 40 and 47 mo exposure, showed slight age-related changes and increasing difference between control and exposed animals with duration of exposure. These two changes are suggestive of bronchitis, similar to the "industrial" bronchitis of mine workers. Along with a suggestion of change in the dynamic pulmonary compliance values, these findings indicate changes in the distal airways of the dogs' lungs.

The most notable pulmonary lesions observed in dogs exposed for up to 4 yr are vesicular emphysema, peribronchiolitis and focal pneumoconiosis. The degree of pulmonary interstitial fibrosis (other than that associated with the focal pneumoconosis-related changes) was slight, and no dose response was evident. This is in contrast to the marked pulmonary fibrosis found, in earlier studies, in the lungs of dogs following approximately 3 yr exposure to radon daughters and carnotite ore dust, suggesting a more rapid development of this lesion when radon daughters are combined with carnotite ore dust.

Lesions of the major airways and upper respiratory tract, when present, were minimal in severity. Pulmonary vesicular emphysema, present in all but one of the examined dogs, was dose-related, in that

TABLE 5. Degree of Severity of Selected Pulmonary Changes Resulting from Chronic Inhalation Exposure of Dogs to Uranium Ore Dust.

		Degree of Severity(a)			
		Pulmonary Vesicular Emphysema			
Dog Number	Duration of Exposure, mo	Subjective(b)	Morphometric(c)	Focal Pneumoconiosis(b)	Peribronchiolitis(b)
1670	12	1	57	2	1
1675	12	1	59	2	1
1679	12	1	64	1	0
1467	20	1	76	2	0
1462	26	1-2	97	3	2-3
1469	26	1-2	60	2-3	1
1468	37	1	58	2-3	1
1493	37	1-2	77	2-3	1
1499	37	1-2	90	2-3	1
1473	48	2	90	2	1
1464	48	2	87	2-3	1
1478	48	2	82	2	1

(a) Code: 1 - Barely perceptible
2 - Readily apparent
3 - Obvious change

(b) Subjective grading based on light microscopy evaluation (all lung lobes)

(c) Mean chord length across alveoli (μm, morphometric evaluation; right diaphragmatic lobe only)

it was present to only a slight degree in dogs exposed for less than 3 yr, increasing in severity thereafter.

Morphometric measurement data confirmed the value of the histopathologic system for grading the degree of emphysema.

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• Toxicology of Krypton-85

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Technical Assistance: R. N. Eichner and A. W. Endres

The purpose of this research is to obtain biological data to supplement earlier evaluations of the hazards of ^{85}Kr exposure. The studies include both short-term and chronic exposure of rats, dogs and sheep to determine tissue distribution and retention kinetics for metabolic modeling purposes. We have included dose-effect studies in rats exposed acutely as newborns or chronically for most of their life span to identify tissues at risk and determine tumorigenic potency.

This report presents progress in two ongoing dose-effect studies employing male and female Wistar rats exposed to graded dose levels of ^{85}Kr. The first study involves adult rats exposed continuously (24 hr/day, 7 days/wk) for 808 days to ^{85}Kr atmospheres equivalent to 10^2, 10^3 or 10^4 times the maximum permissible concentration in air. ($MPC_a = 3 \times 10^{-7}$ μCi ^{85}Kr/ml for areas of uncontrolled access or residential areas.) In the second study, newborn rats were exposed to ^{85}Kr <48 hr after birth to investigate the tumorigenic effect of skin-surface doses of approximately 958, 2328 or 4738 rad. Effects on life span, weight gain and other preliminary observations of toxicity, including tumorigenesis, were presented for both studies in Annual Reports for 1975 through 1980.

In the chronic study, ^{85}Kr exposures were discontinued after 808 days (for budgetary reasons). Approximately 90% of the original 400 rats (100 rats per group, including a group of room-air controls) were dead when exposures were terminated. The surviving rats were transferred from the exposure chambers to standard animal quarters, where they were weighed each month, radiographed, necropsied, and otherwise processed in the same manner as during the exposure period. Malignant lesions observed when tissues from necropsied rats were examined for histopathologic changes are summarized in Table 1.

TABLE 1. Summary of Tumorigenic Response in Rats Chronically Exposed to ^{85}Kr.

Exposure Concentration, μCi/ml[a]	3×10^{-3}	3×10^{-4}	3×10^{-5}	Control
	Cumulative Radiation Dose, rad/yr[b]			
Skin Surface	1465	141	18	1.7
Lung	28	3	0.3	0
Tumor Site	No. Malignant Tumors/Dose Group			
Skin	1	0	0	2
Subcutaneous Tissue	3	10	8	7
Lung	2	3[c]	1	1
Adrenal	1	2	1	3
Skeleton	0	0	1	0
Mammary Tissue	2	2	3	0
Urogenital Tissue	4	2	0	3
Other Visceral Organs	10	9	8	14
Leukemia[d]	2	2	2	1
Number of Rats/Dose Group	100	100	99	100
Average Survival Time, days	747	721	781	794

[a] Krypton-85 chamber concentration was monitored continuously with an in-line Triton gas counter coupled to a chart recorder. The gas counter was calibrated by comparison with aliquots of the chamber atmosphere, analyzed by liquid scintillation counting.

[b] Skin surface dose was estimated with thermoluminescent dosimeters suspended between cages during exposure. Previously reported skin doses were 2-fold greater due to an error in evaluating the dosimeter reading. Lung dose (beta) was calculated assuming a lung weight of 2.5 g and lung volume of 5 ml. The dose contributed by tissue-bound ^{85}Kr (estimated to be ~3% of total lung dose) was not included.

[c] Diagnosis of one lung tumor remains tentative.

[d] No specific site. Listed because of possible association of leukemia with ^{85}Kr exposure (see text).

The incidence of malignant lesions was not treatment-related in tissues receiving the greatest radiation dose (skin > lung > adrenal), nor in the other organ systems where they were found. The chi-square test for homogeneity, which compared tumor incidence in ^{85}Kr-exposed groups and controls, was not significant at P > 0.1. Nor were differences in tumor incidence among ^{85}Kr-exposed groups significant at P > 0.1. The results demonstrate that continuous exposure of rats to ^{85}Kr concentrations equivalent to 100 to 10,000 times the MPC_a for the general population did not significantly affect life span or malignant tumor incidence. These findings attest to the conservatism of the established limits for occupational and population exposure to ^{85}Kr.

Newborn rats were exposed to ^{85}Kr in two replicate studies, 1 yr apart. The second study was initiated to supplement the data obtained from the limited number of rats (~10 per group) used in the preliminary study. Since the dose groups for the two studies were quite similar (853 vs. 1064; 2342 vs. 2315; 4685 vs. 4790 rad, respectively), the results are combined in Table 2. Although the study is not complete, preliminary results suggest a clear association between ^{85}Kr exposure and skin cancer. Malignant lesions of the epidermis appear to be a specific result of ^{85}Kr radiation, as would be expected from the range of the 0.67-MeV beta emission and the proximity of the target cells, presumably located in the basal-cell layer. In our study, a dose of about 100 rad produced two basal cell carcinomas, a lesion which is extremely rare in the Wistar rat. (None were found in the 399 rats examined in the chronic exposure study.)

The two leukemias found in the newborn irradiated rats are within the range of spontaneous tumor incidence observed in our previous studies. There was no indication of a dose-related increase in incidence of leukemias in either the chronic or acute study. The induction of leukemia, as observed by Kirk (1976) (cited by Harley and Pasternack, Health Phys. 33: 567-575, 1977) in adult guinea pigs exposed to near-lethal doses of ^{85}Kr, was not borne out in the preliminary results from our studies. However, the results of the two studies may not be directly comparable because of experimental differences, e.g., difference in species, age when irradiated, magnitude (and other characteristics) of the radiation insult.

TABLE 2. Malignant Skin Tumors in Rats Exposed as Newborns for 6 hr to ^{85}Kr Atmospheres.

Mean Skin Dose, rad ± SD[a]	Mean Exposure Concentration, μCi/ml ± SD	Number of Rats Examined[b]	Number of Rats with Skin Carcinoma				
			Basal Cell	Squamous	Sebaceous	Anaplastic	Leukemia[c]
958 ± 100	5.2 ± 0.5	12 (32)	2	0	0	0	1
2328 ± 240	12.7 ± 1.3	20 (33)	9	3	0	1	1
4738 ± 490	25.9 ± 2.7	23 (25)	13	6	2	2	0
Control		13 (33)	0	0	0	0	0

(a) Dose was estimated from thermoluminescent dosimeter measurements on rat "phantom" placed in exposure chamber.

(b) Total number of rats per dose group shown in parentheses. Pathologic examination is not yet complete.

(c) Leukemias are listed because of possible association with ^{85}Kr exposure (see text).

• Toxicity of Thorium Cycle Nuclides

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Technical Assistance: F. N. Eichner and A. W. Endres

The purpose of this project is to investigate the biological hazards associated with uranium-thorium breeder fuels and fuel recycle process solutions. Initial studies will emphasize the metabolism and long-term biological effects of inhaled ^{233}U-^{232}U nitrate and oxide fuel materials and of ^{231}Pa, a major, long-lived, radioactive waste product.

This report presents progress in two ongoing studies, also discussed in previous Annual Reports, entitled "Disposition and Late Effects of Inhaled $^{233}UO_2(NO_3)_2$ and $^{232}UO_2(NO_3)_2$ in Rats" and "Early Disposition of Inhaled ^{231}Pa Citrate." Also reported are preliminary results from a study of early (1 hr after exposure) translocation of intratracheally instilled actinide nitrates in rats. Earlier progress reports for these studies are presented in PNL Annual Reports for 1975 through 1980.

Long-term pulmonary retention of graded doses of inhaled $^{233}UO_2(NO_3)_2$ and $^{232}UO_2(NO_3)_2$ was determined in male Wistar rats held for lifespan study of biological effects. Uranium retention in the lungs of individual rats is shown in Figures 1 and 2, along with average values and standard deviations for retention in groups of rats (five per group) sacrificed periodically during the initial 200 days after inhalation exposure. Pulmonary retention of ^{233}U (Figure 1) after the first week appeared to be dose-related; possibly due to the ~60-fold range in initial lung burden (ILB) or the ~5-fold range in particle size (AMAD). Clearance during the initial week was influenced as expected, i.e., the high dose group with the largest particle size (2.12 μm AMAD; Table 1) cleared an amount of ^{233}U equivalent to 76% of the ILB during this period compared to only 30 to 63% in the two lower dose groups, which had AMADs in the submicron-size range. The larger particles were, presumably, deposited in the upper airways and cleared rapidly by mucociliary processes.

Results for ^{232}U (Figure 2) are incomplete except for the highest dose group, where rats deposited 53 nCi ILB. Clearance from the high-dose animals was unique in that ^{232}U equivalent to 86% of the ILB was cleared with a half-life of 44 days (Table 1). A relatively small fraction, <1% ILB, was retained with an indeterminately long half-life. Partial data for rats with lower ^{232}U burdens (0.74 or 5.85 nCi ILB; Figure 2) do not exhibit the marked early clearance phase seen in the high dose group. On the contrary, the data suggest a more prolonged retention of ^{232}U than those in Figure 1 and Table 1 for ^{233}U. Since the particle size of all ^{232}U aerosols was very similar, the difference in clearance kinetics may be due to differences in radiation dose to the lung or to subtle physical/chemical differences in the exposure solutions. Tissue analyses now in progress may help resolve these discrepancies.

Male Wistar rats exposed to graded doses of ^{231}Pa citrate aerosols (1, 17 or 56 nCi ILB) have been observed for 550 days after exposure. One lung tumor has been observed in the 56-nCi group in this ongoing study, a finding which is comparable to results with other actinide nitrates we have investigated. Major tissues that retained ^{231}Pa are shown in Figure 3. Protactinium-231 was retained in the lung with a half-life of 71 days, which is comparable to values for ^{233}U in Table 1. Inhaled ^{231}Pa was translocated mainly to the skeleton, where it is retained tenaciously. Based on this deposition pattern, the lung and skeleton are expected to be the primary target tissues for long-term biological effects.

Female Wistar rats were anesthetized and prepared with bile duct and bladder cannulae before intratracheal instillation of various actinide nitrate solutions in a study to determine the magnitude of early (1 hr after exposure) biliary and urinary actinide excretion. This study was initiated in an attempt to explain unexpected low values for initial deposition of uranium in the lungs of rats exposed to ^{233}U and ^{232}U nitrate aerosols. Preliminary results (Table 2) show that only a small fraction of the actinide dose was

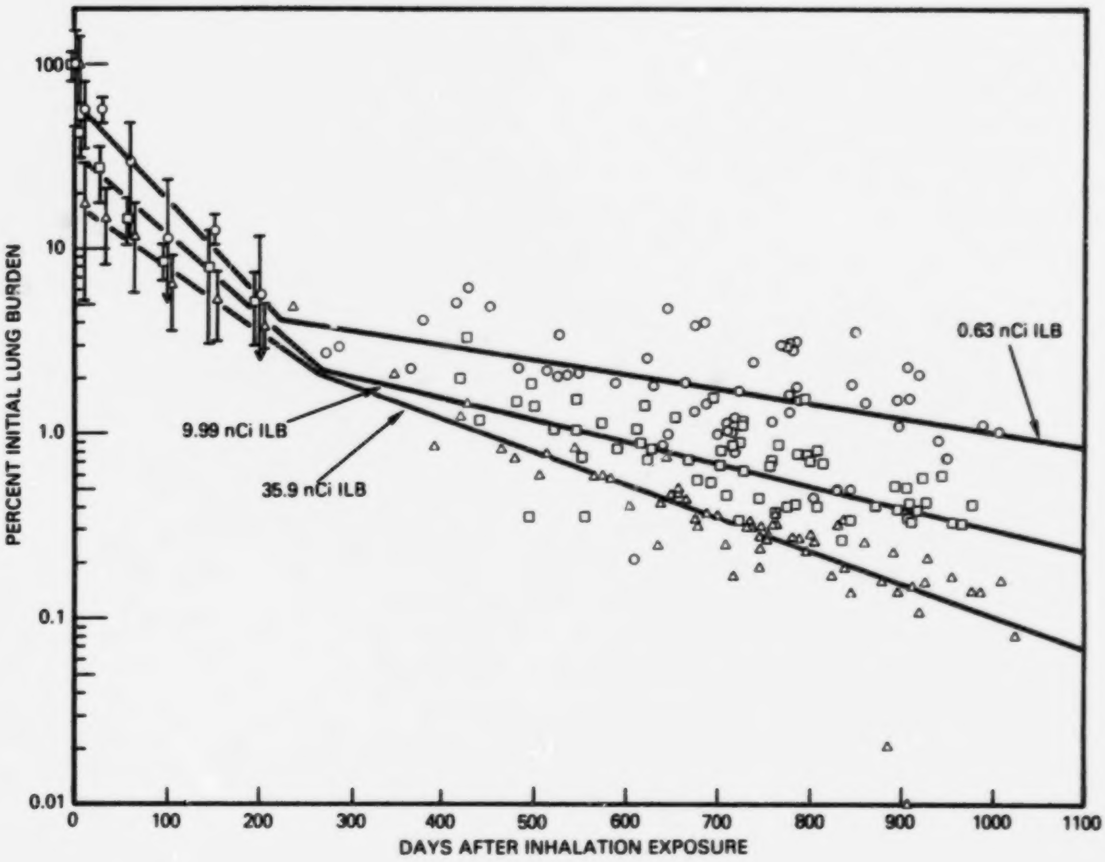

FIGURE 1. Long-Term Retention of Inhaled $^{233}UO_2(NO_3)_2$ in Rat Lungs.

excreted in bile and urine during the initial hour after exposure, although significant amounts of some radionuclides were translocated to tissues by other processes during this time period (Table 3). Uranium-233 was extensively translocated from the lung to kidney, carcass and pelt within 1 hr after instillation. The relatively high deposition in kidney, corresponding to about 26% of the instilled dose and equivalent to 75 µg of uranium, appears to represent tissue-bound ^{233}U that is not readily cleared by urinary excretion. Animals that received a 60-fold-lower ILB of ^{233}U, inhaled as an aerosol, retained (in kidney, 7 days after exposure) an amount equivalent to only 3% of the ILB. The amount retained in kidney immediately after inhalation was not determined; however, cumulative urinary excretion on the first day after exposure accounted for 30% of the ILB. Normal kidney function may have been impaired in the surgically prepared, intratracheally instilled animals; or the increased kidney deposition of ^{233}U may be indicative of uranium metal toxicity associated with the larger instilled dose (2.84 µCi, equivalent to 0.3 mg ^{233}U). Additional studies are planned to help elucidate this problem.

Intratracheally instilled ^{231}Pa was translocated principally to liver, pelt and tissue residuum (Table 3). It is noteworthy that >10-fold more ^{231}Pa was deposited in the tissue residue (which included blood and gastrointestinal tract) than in bone. The amount in skeleton agrees fairly well with data for inhaled ^{231}Pa (Figure 3). Analysis of soft tissues from these animals is now in progress to verify the unexpectedly high accumulation in these tissues.

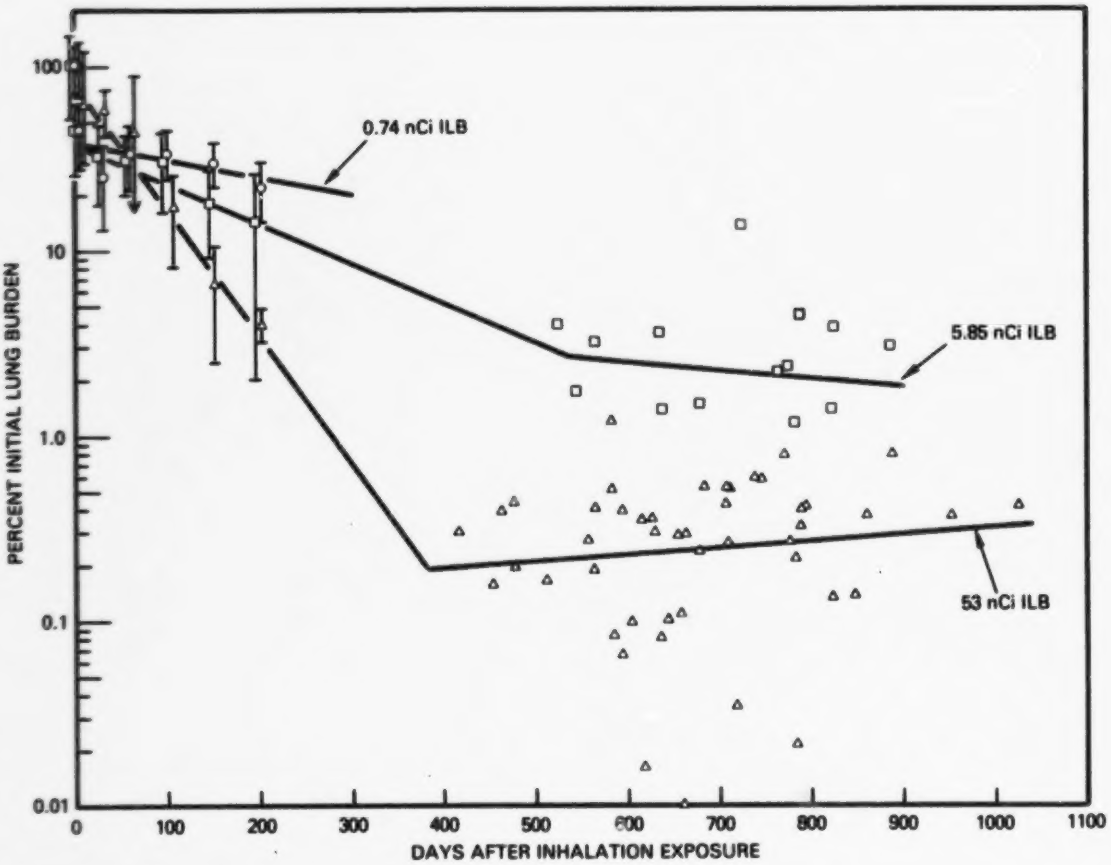

FIGURE 2. Long-Term Retention of Inhaled $^{232}UO_2(NO_3)_2$ in Rat Lungs.

TABLE 1. Comparison of Lung Clearance of Inhaled $^{233}UO_2(NO_3)_2$ and $^{232}UO_2(NO_3)_2$ in Rats.

	Aerosol Characteristics		Clearance Parameters for Specified Retention Intervals[a]					
			0-7 days		7-200 days		>200 days	
Initial Lung Burden, nCi ± SD	AMAD, μm	σ_g	$T_{1/2}$, days[b]	% ILB	$T_{1/2}$, days	% ILB	$T_{1/2}$, days	% ILB
$^{233}UO_2(NO_3)_2$								
0.63 ± 0.36	0.479	1.71	~1	30	56	64	384	6
9.99 ± 1.89	0.994	1.95	~1	63	68	33	255	4
35.9 ± 14.2	2.12	2.19	~1	76	86	18	167	6
$^{232}UO_2(NO_3)_2$								
0.74 ± 0.2	0.336	1.82	~1	NA[c]	316	38	NA[c]	NA[c]
5.85 ± 2.8	0.433	1.95	~1	51	122	44	664	5
53 ± 16	0.356	2.24	~1	14	44	86	Increasing Function[d]	0.2-0.4

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[a] Data were fit with single exponentials ($Y = ae^{bx}$) for the intervals 7-200 days after exposure and time interval greater than 200 days.

[b] Immediate lung clearance (0-7 days) was assumed to occur principally by absorption and mucociliary processes with a half-life of about 1 day.

[c] Data not yet available

[d] Data suggest an increase in ^{232}U deposition in lung after 200 days. This trend is not considered to be real, but is most likely due to errors in the data.

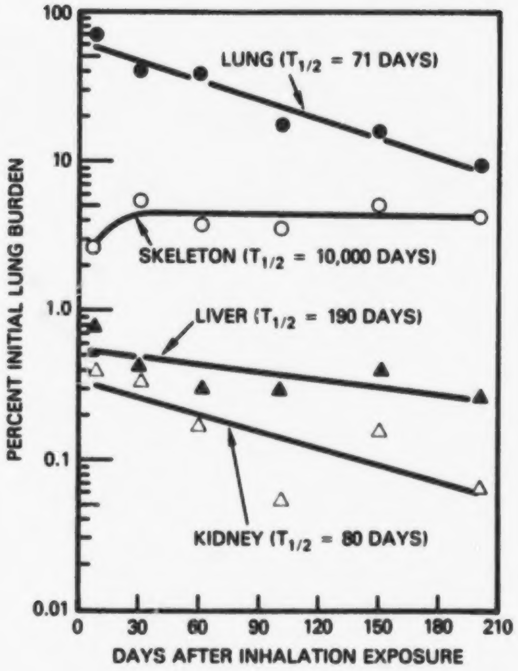

FIGURE 3. Retention of Inhaled ^{231}Pa Citrate in Rats. (Values are the average of retention in 15 rats [combined for the three dose levels, 1, 17 and 56 nCi ILB, respectively], expressed as % ILB ± SD.

TABLE 2. Biliary and Urinary Excretion of Intratracheally Instilled Actinide Nitrate Solutions in the Rat. (Values are averages of retention in five rats expressed as % dose/interval, with four consecutive samples collected at 15-minute intervals after instillation.)

	Percent of Dose/15 min Interval							
	Bile				Urine			
Nuclide	0-15	15-30	30-45	45-60	0-15	15-30	30-45	45-60
$^{233}UO_2(NO_3)_2$	0.0113	0.0162	0.016	0.0157	0.182	0.315	0.125	0.142
$^{238}Pu(NO_3)_4$	0.00042	0.000436	0.000402	0.00035	0.00292	0.0101	0.00895	0.0138
$^{231}PaO_2(NO_3)_x$	ND	ND	ND	ND	ND	ND	ND	ND
$^{239}Pu(NO_3)_4$	0.000179	0.000184	0.000106	0.000829	0.0305	0.0415	0.0421	0.0509
$^{241}Am(NO_3)_3$	0.0000203	0.0000302	0.0000579	0.0000398	0.000669	0.00308	0.00417	0.00915

ND = None detected

TABLE 3. Distribution of Actinide Nitrates in Rat Tissues 1 hr After Intratracheal Instillation. (Values are averages of retention in five rats, expressed as % dose. The rats the same animals described in Table 2.)

Nuclide	Lung	Liver	Kidney	Head	Pelt	Skeleton	Tissue(d) Residuum	Total
$^{233}UO_2(NO_3)_2$	21.4	0.844	26.2	(a)	5.08	22.4	17.2	93
$^{238}Pu(NO_3)_4$	135	0.783	0.185	(a)	0.628	1.96	6.98	146
$^{231}PaO_2(NO_3)_x$	76.3	1.65	0.54	(b)	3.5	1.57	24.2	108
$^{239}Pu(NO_3)_4$	72.9	0.029	0.00811	1.15	0.13	0.84(c)		75
$^{241}Am(NO_3)_3$	84.2	0.569	1.47	6.17	0.408	0.775(c)		94

(a) Head included with skeleton and tissue residuum.

(b) Head discarded

(c) Skeleton and tissue residuum combined.

(d) Tissue residuum includes remaining visceral organs, blood and skeletal muscle.

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• Fetal and Juvenile Radiotoxicity

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This project is directed at obtaining detailed comparative information on the deposition, distribution, retention, and toxicity of radionuclides in the prenatal and juvenile mammal. Because quantitative data cannot necessarily be extrapolated to man, our emphasis is directed toward establishing patterns, phenomenologic interactions, and relationships which will be useful in determining appropriate exposure levels for the rapidly growing infant or child, and for pregnant women.

Recent results demonstrated that injection of pregnant rats with ^{239}Pu had the greatest effect on longevity and bone-tumor incidence of the offspring when exposure occurred at 19 days of gestation (dg); less effect at 15 dg and the least effect at 9 dg. Exposure of pregnant rats to ^{239}Pu during late gestation was shown to adversely affect their ability to rear offspring and partially explains the greater incidence of bone tumors in rats reared by foster-control mothers after prenatal exposure to plutonium, relative to those reared by their own mothers. Ongoing distribution studies are providing data which confirm our tentative explanation that marked variations in the anatomic distributions of bone tumors, with age at the time of injection, were attributable to age-related differences in ^{239}Pu microdosimetry and concentrations among skeletal components. Other studies, using a placental perfusion technique, have demonstrated that intravenous injection of ^{239}Pu in pregnant guinea pigs leads to a marked decrease in maternal blood flow to the placenta.

The data on the incidence of tumors and other lesions from our completed experiment which compared the late effects of monomeric plutonium exposure in prenatal, neonatal, weanling, and adult rats were adjusted on the basis of survivorship. This transformation demonstrated differences among groups in the temporal development of tumors but did not change our previous conclusions: perinatal rats are less sensitive to ^{239}Pu carcinogenesis than adults on the basis of administered dose, but are more sensitive on the basis of radiation dose to the skeleton. Prenatal exposure at 19 days of gestation (dg) leads to an increase in tumor incidence, even though the cumulative radiation doses to the skeleton are below 80 rad. To provide a basis for comparison with data from a subsequent experiment, these data are presented as the points indicated by "A" in Figure 1.

The protocol for another experiment on late effects of ^{239}Pu exposure at 9, 15, or 19 dg, using doses within the same dose range as those used in the previous study, was described in a previous Annual Report (1978). Longevity of the offspring of both sexes was significantly decreased by the highest dose administered at 19 dg. Survival was less affected in those ex-

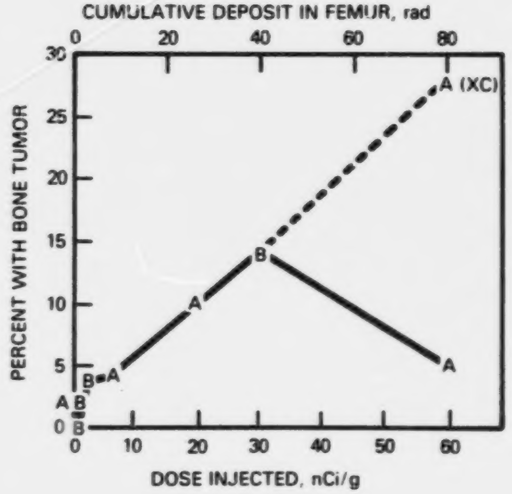

FIGURE 1. Bone-Tumor Incidence in Offspring from Rats Exposed to Monomeric ^{239}Pu at 19 Days of Gestation. Data are combined from two separate experiments, identified as A and B; point A [XC] relates to prenatally exposed offspring reared by unexposed dams.

posed at 15 dg and was unaffected in those exposed at 9 dg. Based on histopathologic evaluations, bone-tumor incidence appears to be increased by the highest dose (30 μCi/kg) at each gestational period, and is highest in those exposed at 19 dg. Bone-tumor incidences in offspring exposed at 19 dg (presented as the points indicated by "B" shown in Figure 1) are consistent with those in the previous experiment.

Bone-tumor incidence in prenatally exposed offspring (Figure 1) increases progressively with increased doses through 30 μCi/kg (40 rad to the skeleton), followed by a lower incidence in offspring from females exposed to the highest dose, 60 μCi/kg. As was reported previously, bone-tumor incidence in offspring from females exposed at this dose level, reared by foster-control dams (indicated in Figure 1 as A [XC]), was higher than in those which remained with their exposed dam, even though they received a somewhat lower cumulative radiation dose. These findings may be attributable to a phenomenon associated with fostering, per se, but since this result was unexpected, all appropriate fostering combinations were not included in the original experimental design. Alternatively, the findings may reflect repression of bone-tumor development in offspring reared by exposed dams as a result of a growth depression produced by nutritional impairment. If this is true, incidence in the A [XC] group may be a more accurate measure of incidence without radiogenic perturbation.

In an attempt to replicate and extend previous findings, we have started an experiment to evaluate the interactions suggested above. Using incremental exposure blocks, pregnant rats were injected with 60 μCi/kg ^{239}Pu or a citrate solution (control) at 19 dg, followed by appropriate neonatal fostering of offspring to form six experimental groups.

Prenatal Exposure	Postnatal Treatment	Group Designation
Control	Not fostered	C
Control	Fostered to control	C-C
Control	Fostered to exposed	C-X
Exposed	Not fostered	X
Exposed	Fostered to control	X-C
Exposed	Fostered to exposed	X-X

Subgroups of 60-70 offspring, each containing no more than two males and two females from a litter, were kept for long-term studies. (The offspring ranged from 1 to 1½ years of age on 10/1/81.) The remaining offspring, together with animals from additional identically exposed litters, were sacrificed at intervals to obtain data on plutonium distribution for dosimetry. Initial growth curves for male offspring are presented in Figure 2. It is obvious that the exposed dams had a reduced ability to rear offspring, as indicated by the depressed growth curves for all groups of offspring nursed by exposed dams. Moreover, growth curves for prenatally exposed offspring reared by control dams are similar to those of control offspring reared by their own or by control foster dams.

Additional studies on ^{239}Pu distribution and dosimetry were performed to supplement those included in the long-term experiments, discussed above, and to extend previously observed age-related differences. In particular, data were needed for times shortly after administration to explain etiologic interactions. These studies have been completed and radioanalytic data are being analyzed; autoradiographs have been prepared and examined from only a small fraction of the specimens. Selected comparisons will be used to illustrate some salient patterns; modeling is still in progress.

Hepatic incorporation was rapid in adults (Figure 3) and the percent of administered dose in the liver at 1 day after injection was approximately 23%. The maximum value was reached more slowly and the percent of dose deposited in the weanling liver (14%) was substantially less than that in the adult. There was markedly higher hepatic incorporation (46%) in newborns; the rise to the 1 day value was slower than in the older animals, and the fraction in liver further increased by 3 days.

Confirming previous observations, the percent of dose in the femur at 1 day was substantially higher in weanlings than in adults, although it was much lower in newborns. Both adults and weanlings attained maximal deposition by about 3 days after exposure, and this level remained essentially constant through 28 days after exposure. The lower deposition level in newborns was maintained through 7 days, followed by a progressive rise, which reflected the continued translocation of plutonium from the liver.

Data are presented in Figure 3 for the bisected mandible (one-half) which was taken for radioanalysis. Early incorporation in the weanling mandible was 3 times as great as the percentage of the dose incorporated in the adult. Reflecting the proportionately greater size of the mandible, relative to the femur in the

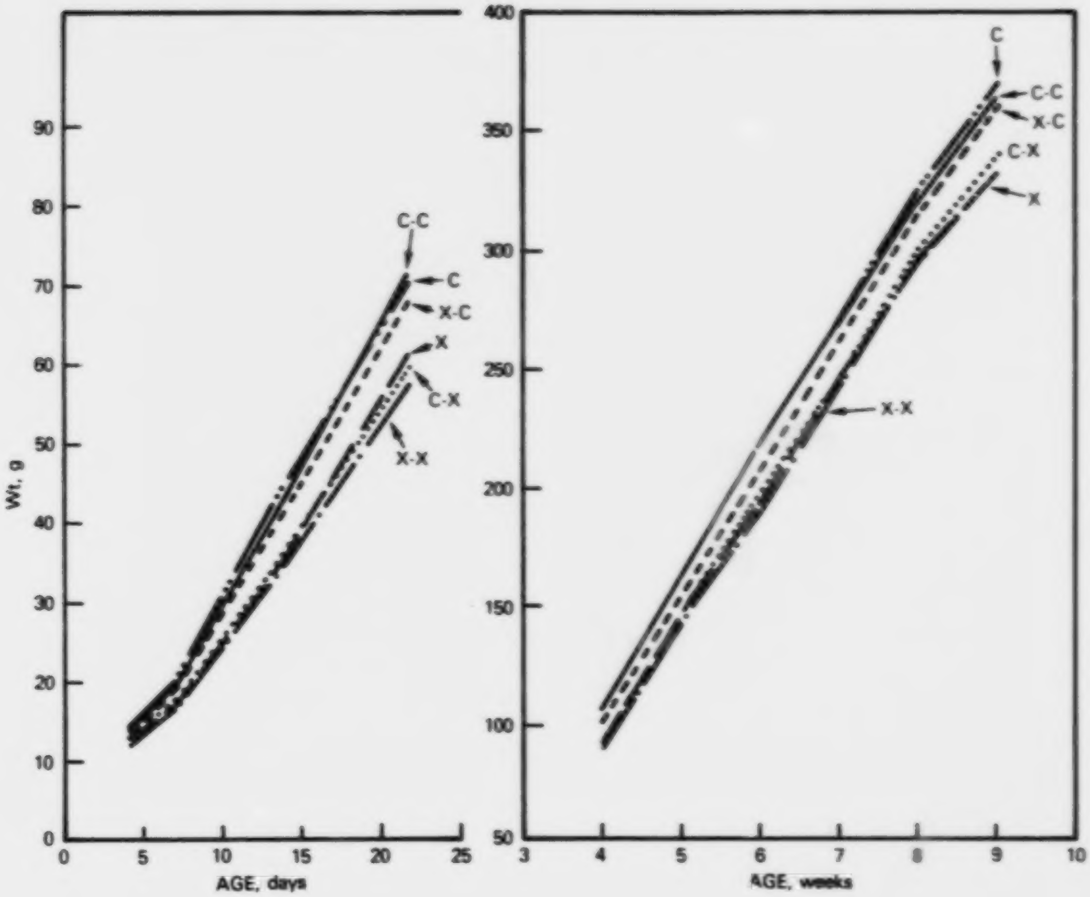

FIGURE 2. Relationships Between Prenatal ^{239}Pu Exposure and Neonatal Fostering on Growth of Male Offspring. (See text for identification and description of groups.)

newborns, 1- and 3-day incorporation values were markedly greater than in adults, and approximated those in the weanlings as ossification progressed at later time periods.

Plutonium concentrations throughout the skeleton (Table 1) reflected growth rate differences within and among age groups and differences in incorporation which, in turn, is affected by developmental and metabolic events. Among the several types of analyses being performed, is an examination of the relative concentrations of plutonium in representative skeletal components in the 3 age groups (Table 1). In adults, the concentrations in the femur and vertebral column were similar at 1 day, but were more than twice those in the bones of the head (calvarium and mandible); all values were slightly increased at 14 and 28 days after exposure. Reflecting accretion and growth in the weanlings, all concentrations were slightly greater at 3 days than at 1 day after exposure but progressively decreased thereafter. The concentrations in the vertebrae, calvarium and mandible were similar to each other but were substantially less than that in the femur. As a result of the high but disproportionate growth rates of the skeletal elements in the newborn, all concentrations markedly decreased with time subsequent to 1 day, but the fractional decreases were greatest and the final concentrations were significantly

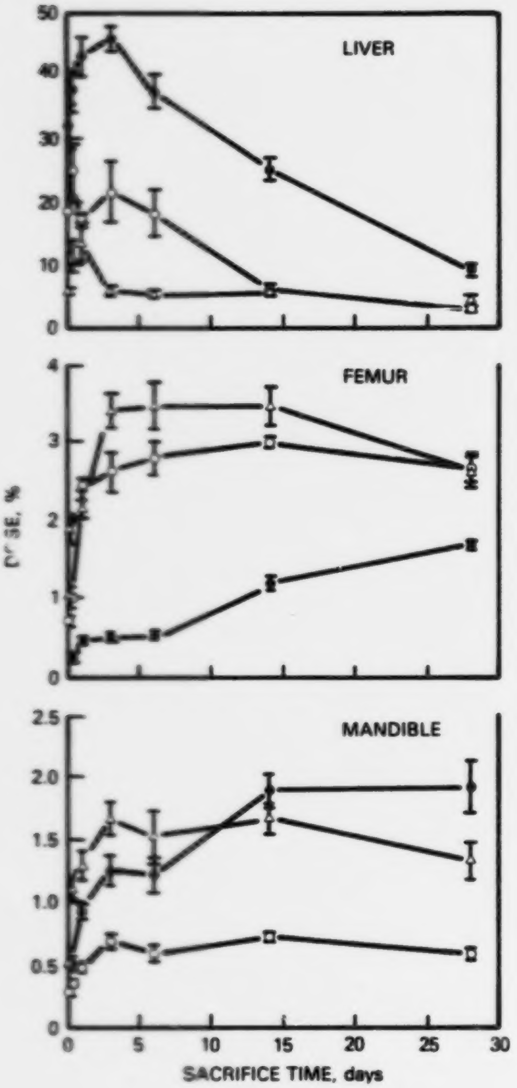

FIGURE 3. Content (% Dose) of ^{239}Pu in Representative Structures after Injection of Newborn (●), Weanling (Δ) and Adult (O) Rats.

TABLE 1. Relative Concentration[(a)] of ^{239}Pu in Representative Skeletal Components at Selected Times After Administration to Newborn, Weanling and Adult Rats.

	Days After Administration		
	1	14	28
Newborn			
Calvarium	1.3	0.76	0.59
Mandible	1.4	0.80	0.42
Vertebrae	1.6	0.59	0.31
Femur	1.0	0.38	0.28
Weanling			
Calvarium	0.73	0.66	0.38
Mandible	0.56	0.52	0.34
Vertebrae	0.72	0.37	0.31
Femur	1.0	0.71	0.45
Adult			
Calvarium	0.40	0.56	0.59
Mandible	0.36	0.47	0.42
Vertebrae	0.95	1.2	1.1
Femur	1.0	1.2	1.1

(a) Standardized for each age group to a concentration of 1.0 in the femur at 1 day postinjection.

lower in the femur and vertebrae than in the bones of the head. These age-dependent changes in concentration patterns are of particular interest since they will provide a dosimetric explanation for our reported findings that the anatomic distribution of bone tumors differs with age at exposure (predominantly in the heads of perinatally exposed animals and in the vertebral columns and extremities of adults).

The relatively low fraction of a Pu dose initially incorporated in the femur of newborns has not been satisfactorily explained since there is a large amount of bone surface available for deposition. This may be due to the fact that little ossification has occurred, and it appears that the bone surfaces in the newborn do not fully participate in plutonium deposition. Moreover, there is substantial metaphyseal deposition in autoradiographs of undecalcified femur sections in both weanlings and adults, but the metaphysis is completely devoid of plutonium activity at early times after injections in the newborns.

Our studies have demonstrated that only a small amount of the plutonium administered to the pregnant rodent accumulates in the fetus. To better quantitate this result, we used a placental perfusion technique to directly measure the clearance of plutonium from dam to fetus in the guinea pig.

As reported in last year's Annual Report, the amount measured was 2.3 ± 0.6 µl/min (mean ± SE), the lowest measured for any substance using this technique. Since clearance appeared to be influenced by a decrease in placental blood flow, the data from concurrently measured tritium clearances (T_{MF}) were analyzed in further detail (Table 2). Tritium clearance (0.52 ± 0.09 ml/min at an umbilical flow rate of 2.5 ml/min) was linearly related to umbilical flow rate in only five of 10 perfusions, but no such relationship was present in the remaining five. Even when present, the linear relationship between T_{MF} and umbilical flow did not persist throughout the perfusion period. Adjustment of the plutonium clearances to those which would have been expected if this decrease in maternal blood flow had not occurred still indicated an extremely low plutonium clearance.

The changes in placental flow resulting from plutonium exposure were unexpected, and the experiments were not designed to quantitate kinetics under conditions of changing maternal blood flow. Therefore, an additional series of experiments were initiated and are continuing. These experiments have ruled out the possibility that the citrate vehicle in which the plutonium was administered was responsible for the effect on maternal blood flow to the placenta. At the level used (30 µCi/kg), plutonium appears to produce a progressive decrease in tritium clearance, starting approximately 8 min after injection. The T_{MF} remains at a low level throughout the 30-min to 1-hour duration of the experiment. The doses and the mass of plutonium administered in these experiments were relatively large (approximately 500 µg/kg); however, no radiogenic or chemical mechanism for such a rapid decrease in blood flow is apparent at the present time. These experiments are being extended to establish the levels at which these blood-flow effects occur and the duration of effects.

TABLE 2. Values from Regression Analyses[a] of Tritiated Water Clearance from Dam to Fetus in Pu-Dosed Guinea Pigs.

Dam Number	Placenta Number	Intercept, a	Slope, b	Probability	Correlation Coefficient	Estimated Tritium Clearance[b]
1	1	0.013	0.276	<0.001	0.81	0.65 ± 0.02
2	1	NS	NS	>0.20	NS	--
2	2	0.213	0.174	<0.001	0.84	0.65 ± 0.04
3	1	-0.072	0.140	<0.001	0.85	0.28 ± 0.01
4	1	NS	NS	>0.30	NS	--
4	2	NS	NS	>0.30	NS	--
5	1	NS	NS	>0.30	NS	--
5	2	0.035	0.177	<0.001	0.87	0.44 ± 0.02
6	1	NS	NS	>0.20	NS	--
6	1	0.055	0.202	<0.001	0.92	0.56 ± 0.03

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[a] $Y = a + bX$ where Y = tritium clearance and X = perfusion rate.

[b] Tritium clearance ± SE (ml/min) at a perfusion rate of 2.5 ml/min as estimated from the regression of tritium clearance on perfusion rate.

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• Gut-Related Radionuclide Studies

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Other Investigators: B. M. Miller and J. L. Ryan

Technical Assistance: K. I. Cousineau, K. D. Fisk, and N. M. Goranson

This project is concerned with the behavior of radioactive materials that may be ingested as a consequence of a reactor accident, unavoidable occupational exposure, or after release to the environment and incorporation into the food chain. Current emphasis is directed toward evaluating hazards from ingested actinides as a function of animal age, species, nutrition, and diet, or chemicophysical state of the actinide. It is also concerned with the behavior of actinides that are inhaled and pass through the gastrointestinal (GI) tract after clearance through the lungs.

Recent observations indicate that the influence of chemical form on plutonium absorption observed at high mass levels does not occur at low mass concentrations. For example, at doses of 0.6 µg/kg there was no difference between absorption of the carbonate, citrate or nitrate forms of plutonium. However, at 1.5 mg/kg, the citrate was absorbed in quantities 30 times higher than the nitrate. The opposite effect occurred for neptunium GI absorption. The low-specific-activity isotope, ^{237}Np, gavaged at doses of 22 or 43 mg/kg, was absorbed in quantities from 30 to 50 times greater than the high-specific-activity neptunium isotopes, ^{235}NP and ^{239}Np, at doses that were 1×10^6 or 2×10^8, respectively, less than that of ^{237}Np.

We have also demonstrated that materials such as citrus fruit juices and calcium, as well as drugs that affect GI function (such as aspirin and DTPA), markedly influence GI absorption of plutonium. Such studies provide evidence that diet and nutritional state should be considered in establishing safe limits for radionuclides that may be ingested.

Effects of Chemical Form and Plutonium Concentration on Gastrointestinal Absorption by Adult or Neonatal Rodents

From previous studies, it was concluded that 10 times more plutonium is absorbed from the GI tract when administered as the citrate than when administered in less soluble forms, such as the nitrate or carbonate. To determine the importance of compound form at very low plutonium concentrations, we compared the absorption of ^{239}Pu from relatively concentrated solutions in 5% citrate or 0.01 M nitric acid, with absorption of mixed $^{237/239}$Pu at very low concentrations in similar citrate or nitrate solutions.

Groups of adult, male Swiss-Webster mice received 0.25 ml of either the ^{239}Pu or $^{237/239}$Pu preparations intragastrically through polyethylene tubing. Litters of 2-day-old rats were divided approximately equally and received 0.1 ml of solutions of either high or low concentrations of plutonium citrate, carbonate or nitrate. All animals were killed at 7 days after gavage. Those given ^{239}Pu were measured for their alpha radioactivity. Those given $^{237/239}$Pu were counted for the photon emissions of ^{237}Pu, both in a whole-body counter and in a deep-well gamma counter.

Results obtained after gavage of adult mice are summarized in Table 1. The marked difference in absorption of ^{239}Pu citrate and nitrate, fed at high mass levels, was not observed when much lower mass levels of $^{237/239}$Pu were fed; nor was there a significant difference between absorption of the citrate and the less-soluble Pu carbonate.

Our previous comparison (Annual Report, 1980) of the absorption of ^{237}Pu and ^{239}Pu nitrate demonstrated that absorption was inversely related to the mass of Pu administered. The data in Table 1 support that observation.

We also found that mass influenced absorption in neonatal rats (Table 2), although to a lesser extent than in adult animals. Chemical form also affected absorption in neonatal rats at high Pu doses; retention after gavage of ^{239}Pu citrate was about three times that after ^{239}Pu nitrate. At lower mass levels, no such effect was observed.

TABLE 1. The Effect of Concentration and/or Chemical Form on Gastrointestinal Absorption of Plutonium by Mice.

Pu Isotope	^{239}Pu		$^{237/239}$Pu		
Pu Compound	Citrate	Nitrate	Citrate	Nitrate	Carbonate
Activity Administered, μCi/kg	91	91	1	1	1
Mass Administered, μg/kg	1480	1480	0.6	0.6	0.6
No. of Animals	10	10	19	19	5
Time of Necropsy, days	7	7	7	7	7
Tissue	Percent of Gavaged Pu Dose ± SEM				
Carcass, (minus skin and GI tract)	0.2	0.005	0.08	0.07	0.14
Liver	0.08	0.001	0.05	0.01	0.03
Total	0.28 ± 0.03	0.006 ± 0.001	0.13 ± 0.02	0.08 ± 0.02	0.17 ± 0.07

TABLE 2. The Effect of Concentration and/or Chemical Form on Gastrointestinal Absorption of Plutonium by Neonatal Rats.

Pu Isotope	^{239}Pu		$^{237/239}$Pu	
Pu Compound	Citrate	Nitrate	Citrate	Nitrate
Activity Administered, μCi/kg	115	115	0.9	0.9
Mass Administered, μg/kg	1875	1875	0.6	0.6
No. of Animals	7	10	11	6
Time of Necropsy, days	7	7	7	7
Tissue	Percent of Gavaged Dose ± SEM			
Carcass (minus skin and and GI tract)	2.5	0.8	1.6	2.1
Liver	0.3	0.2	0.2	0.3
Total	2.8 ± 0.8	1.0 ± 0.1	1.8 ± 0.1	2.4 ± 0.3
GI Tract and Content	27 ± 6	36 ± 3	62 ± 2	67 ± 1

Effect of Mass on Gastrointestinal Absorption of Neptunium

Neptunium may be the most hazardous nuclear waste product in the distant future (>2000 yr) because of its long half-life isotope ^{237}Np (2.1 x 10^6 yr) and its reactivity in biological systems. Our previous results (Annual Reports, 1973, 1975) indicated that the absorption of ^{237}Np nitrate was about 10 times higher (in adult rats) than that of any other transuranic studied. Earlier data had suggested that absorption was directly related to mass; however we had found the opposite to be true for plutonium (Annual Report, 1980). We therefore conducted experiments to study the GI absorption for ^{237}Np ($T_{1/2}$, 2.1 x 10^6 yr), ^{235}Np ($T_{1/2}$, 396 days), and ^{239}Np ($T_{1/2}$, 2.3 days). Results are summarized in Table 3. There was very little difference in the absorption of ^{235}Np and ^{239}Np, although the mass administered differed by a factor of 180. On the other hand, absorption of either ^{235}Np or ^{239}Np was about 50 times lower than that of ^{237}Np, the mass of ^{237}Np administered being 10^6 to 10^8 times that of ^{235}Np or ^{239}Np. Doubling the mass of ^{237}Np administered almost doubled the amount absorbed, suggesting that chemically toxic effects of the large quantity of neptunium administered may have caused the increased absorption.

Because the quantity of ^{235}Np was limited, only a few rats could be studied; mice were employed to extend the data to more animals and to another species. The ^{235}Np retention in mice could be measured by whole-body counting of the X-ray emissions. A comparison of the mouse and rat data (Table 3) indicates no species difference in absorption.

An experiment with ^{239}Np was performed with neonatal rats for comparison with previous ^{237}Np neonatal rat studies (Annual Reports, 1974, 1975). Those earlier studies showed GI absorption of ^{237}Np in 2- and 9-day-old rats of 0.8 and 1.2%, respectively. The ^{239}Np data in Table 4 show a similar absorption in 2- and 9-day-old rats. Both age groups absorbed at least 25 times the adult value. The percentage retained in the gut was, however, about five times higher for ^{239}Np than the earlier value observed for ^{237}Np. Unlike the case for ^{237}Np, the comparative behavior of ^{239}Np in neonatal and adult rats is quite similar to that of plutonium.

TABLE 3. The Effect of Mass Administered on Gastrointestinal Absorption of Neptunium Nitrate by Adult Rats and Mice.

Np Isotope	^{237}Np		^{235}Np		^{239}Np
Activity Administered, μCi/kg	30	15	18	90	42
Mass Administered, μg/kg	43,000	21,700	0.04	0.13	0.00022
Species	Rats	Rats	Rats	Mice	Rats
No. of Animals	6	6	4	8	10
Time of Necropsy, days	7	7	7	7	3
Tissue	Percent of Gavaged Dose ± SEM				
Carcass (minus skin)	1.5	0.9	0.02	0.03	0.03
Liver	0.24	0.09	0.007	0.01	0.006
Urine	1.0	0.5	0.03	--	0.01
Total	2.74 ± 0.4	1.5 ± 0.2	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01

TABLE 4. The Effect of Age on Gastrointestinal Absorption of ^{239}Np Nitrate by Rats.

Age at Gavage	Adult	9 Day	2 Day
Activity Administered, μCi/kg	50	100	185
Mass Administered, μg/kg	2.2×10^{-4}	4.3×10^{-4}	8×10^{-4}
No. of Rats	10	11	9
Time of Necropsy, days	3	4	4
Tissue	Percent of Gavaged Dose ± SEM		
Carcass (minus skin and GI tract)	0.027	0.87	1.24
Liver	0.006	0.03	0.03
Total	0.03 ± 0.006	0.90 ± 0.05	1.27 ± 0.10
GI Tract (content)	--	59.6 ± 1.3	72.6 ± 8.0

The Effect of Diet, Aspirin and DTPA on the Absorption of ^{238}Pu from the GI Tract of Rats

We previously demonstrated (Annual Reports, 1971, 1980) that the effects of fasting, citric acid, milk and DTPA on the GI absorption of plutonium all resulted in increased absorption. Orally administered DTPA caused enhanced absorption, demonstrated by increased urinary excretion of Pu. Since citric acid enhanced plutonium absorption, it was expected that orange juice might also cause an increase in transport, as it does for lead. Aspirin (acetylsalicylic acid) may also affect transport because of its damaging effects on the mucosa. Calcium deficiency and intravenously administered EDTA causes increased lead toxicity by increasing GI absorption.

To test the effect of orange juice, 2 ml were administered intragastrically to rats that were either fasted for 24 hr before gavage or fed ad libitum. The orange juice gavage was followed immediately by 1 ml of 0.01 M HNO_3 containing ^{238}Pu. The results (Table 5) indicate no increase in absorption due to fasting but a 7-fold increase due to the presence of orange juice in the GI tract.

To test the effect of aspirin, a suspension in 2% methyl cellulose was administered daily by gavage at doses of either 200 or 400 mg/kg. On the third day of treatment, 1.0 ml of 0.01 M HNO_3 containing ^{238}Pu was also gavaged. The results (Table 6) show no increase in liver retention or urinary excretion; however, the amount in bone doubled, causing a 2-fold higher retention in treated animals than in controls. The effect of aspirin was not dose-dependent.

TABLE 5. The Effect of Orange Juice on Gastrointestinal Absorption of ^{238}Pu Nitrate (1.44 μg/kg) by Fed and Fasted Rats.[a]

	Percent of Gavaged Dose ± SEM			
	^{238}Pu Control		^{238}Pu + Orange Juice	
Tissue	Non-Fasted	Fasted	Non-Fasted	Fasted
Carcass	0.02	0.02	0.1	0.11
Liver	0.001	0.002	0.02	0.03
Urine	0.003	0.006	0.017	0.005
Total Absorbed	0.02 ± 0.01	0.03 ± 0.01	0.14 ± 0.05	0.15 ± 0.02

[a] 4 rats per group. see text for details.

TABLE 6. the Effect of Aspirin on Gastrointestinal Absorption of ^{238}Pu Nitrate (40 μCi/kg) by Rats.[a]

	Percent of Gavaged Dose ± SEM		
Tissue	Control	0.05 mg Aspirin/kg	0.1 mg Aspirin/kg
Carcass	0.017	0.049	0.045
Liver	0.005	0.006	0.006
Urine	0.006	0.007	0.007
Total Absorbed	0.029 ± 0.007	0.062 ± 0.01	0.058 ± 0.02

[a] 6 rats per group, see text for details.

To produce a calcium deficiency, a special (calcium-deficient) diet supplied by US Biochemical Corp. (Cleveland, OH) was fed to adult or weanling rats for 2 wk. They were then gavaged with 1 ml of 0.01 M HNO_3 containing ^{238}Pu and maintained on the calcium-deficient diet until sacrifice, 7 days later. The results (Table 7) indicate that absorption and retention of ^{238}Pu was substantially increased. The adult values increased 20-fold, whereas the weanling values increased about seven times.

When DTPA was injected intravenously (0.5 mM/kg) immediately before gavage with 1 ml of 0.01 M HNO_3 containing ^{238}Pu, an increase in urinary excretion (Table 8) was the major indication of an increase in absorption. It was increased about 50 times over that of control rats. Retention in every tissue measured was also reduced by DTPA.

These results demonstrate that plutonium GI absorption may be increased by diet changes or drug treatment. Those effects may be produced directly, by the presence of the nutrient or drug within the GI tract at the same time as the actinide, or they may result from an indirect, systemic effect.

TABLE 7. The Effect of Calcium Deficiency on Gastrointestinal Absorption of ^{238}Pu Nitrate by Adult and Weanling Rats.[a]

	Percent of Gavaged Dose ± SEM			
	Adult		Weanling	
Tissue	Control	Ca Deficient	Control	Ca Deficient
Carcass (minus skin and GI tract)	0.01	0.26	0.06	0.34
Liver	0.002	0.02	0.004	0.04
Urine	0.0008	0.01	0.004	0.12
Total Absorbed	0.013 ± 0.004	0.29 ± 0.04	0.07 ± 0.02	0.53 ± 0.13

[a] 10-12 rats per group, see text for details.

TABLE 8. The Effect of Intravenously Administered DTPA (0.5 mM/kg) on Gastrointestinal Absorption of ^{238}Pu Nitrate by Rats.[a]

	Percent of Gavaged Dose ± SEM	
Tissue	^{238}Pu Control	^{238}Pu + DTPA
Carcass (minus skin and GI tract)	0.017	0.016
Liver	0.005	0.0004
Urine	0.006	1.3
Total Absorbed	0.029 ± 0.007	1.3 ± 0.4

[a] 6 rats per group, see text for details.

• Development of Blood Irradiators

Principal Investigator: F. P. Hungate

Other Investigators: L. R. Bunnell, J. E. Morris, and W. F. Riemath

Over a period of several years, a small, fully portable blood irradiator has been developed for ultimate use in suppressing early rejection of organ transplants in humans. It could also be useful for other medical problems, e.g., treating some forms of leukemia or arthritis. The units have been successfully evaluated in several animal species, resulting in sharply reduced lymphocyte levels and prolonged skin-graft retention. Work during the past year was directed toward development of hardware in anticipation of kidney transplant studies, to be performed in dogs in FY 1982, and identifying whether dose fractionation significantly changed lymphocyte response.

This continuing program to develop a fully portable blood irradiator proceeded along three paths this year. One path was directed toward evaluating the effects of dose fractionation on the cell-mediated immune response. Since only a fraction of the total blood is irradiated at any specific time, knowledge of effect of fractionating the dose is needed.

Effects of dose fractionation on dog blood were tested by exposing the cells to gamma irradiation in vitro and measuring mitogen (concanavalin-A, Con-A) stimulation response, mixed-lymphocyte assays, and cytotoxicity, as measured by ^{51}Cr release. The mitogen-stimulation studies used both isolated lymphocytes and whole-blood cultures, both giving similar data. The data reported here (Figure 1) are for whole blood. They indicate little, if any, difference in response (incorporation of ^{125}I·iododeoxyuridine, ^{125}I·IUdR) to mitogen stimulation between lymphocytes

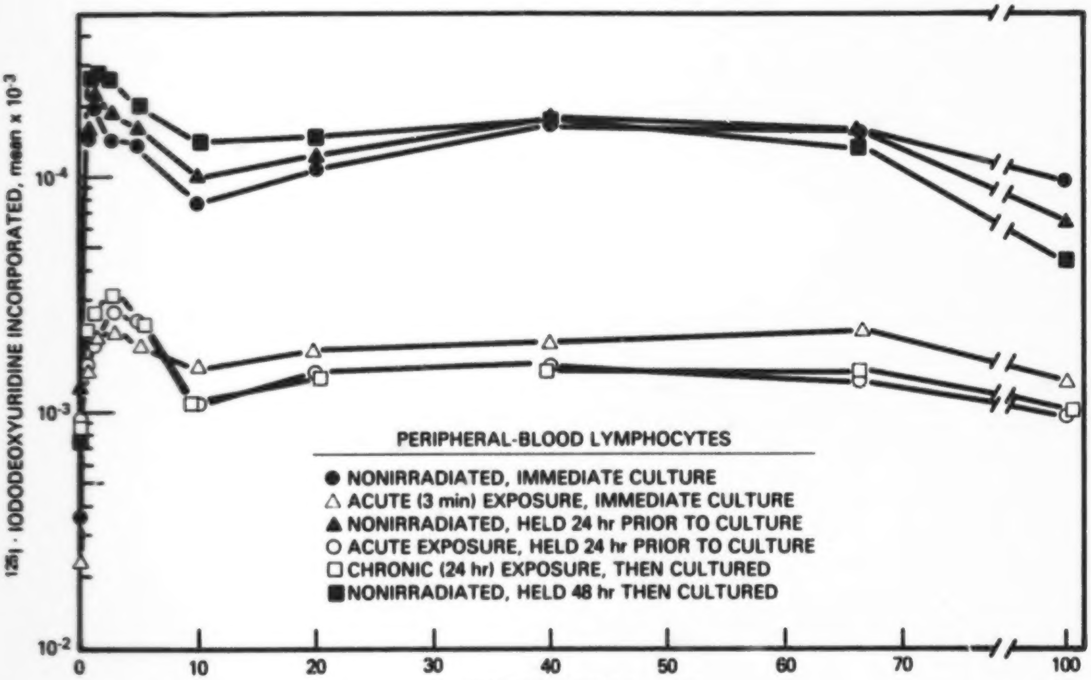

FIGURE 1. Responses to Concanavalin-A in Irradiated and Unirradiated Dog Peripheral-Blood Lymphocytes.

acutely exposed (3 min) and those exposed to essentially the same total dose over 24 hr. However, repair enzymes may not function at the temperature at which these cultures were incubated (20°C), so these data are not yet definitive.

The effect of gamma irradiation on responses of rat peripheral-blood lymphocytes (PBL) to Con-A was greater than on dog blood. Incorporation of ^{125}I·IUdR following exposure to 2400 rad over 24 hr (or 1900 rad in 3 min) was about 3% of that in controls; the incorporation in dog PBL exposed to the same doses was approximately 10% of that in unexposed controls. Dog blood cells maintained the ability to respond fully to the mitogen through 48 hr at 20°; rat blood cells responded fully after 24 hr but had a 90% loss of response after 48 hr.

Procedures for the mixed-lymphocyte and ^{51}Cr cytotoxicity assays have been developed, but no data are as yet available.

The second and third paths were the fabrication of sufficient irradiator units to proceed with kidney transplant studies in dogs in FY 1982, and the development of a more efficient means of activating the units. During the coming year we plan to test the irradiators in six dogs that will receive kidney transplants from unrelated donors. A similar number of tests will be made using nonactivated irradiators. The units for these tests are now available.

The irradiators are produced by neutron activation of ^{169}Tm in a matrix of vitreous carbon. In previous years, we have had great difficulty obtaining a sufficient number of units due to the required amount of time and neutron flux (@ 10^{14} n/cm^2). We have modified the carbon carrier used during activation so that three units can be activated in the same space and time previously required for one unit. This new carrier has not yet been functionally tested, but calculations indicate that heat transfer should be adequate during activation.

Statistical Health-Effects Study

Principal Investigator: E. S. Gilbert

Other Investigators: H. D. Tolley, J. A. Buchanan, and S. Marks

The main purpose of this program is to analyze the mortality of Hanford workers and to determine the effects of radiation exposure in this population. A secondary purpose is to improve methodology for assessing health effects of chronic low-level exposure to harmful agents or substances, particularly in an occupational setting. In the past year we have updated our analyses, submitted papers for publication in the two areas of methodological research, and have interacted with Hanford Environmental Health Foundation staff to improve data collection procedures.

Statistical Health-Effects Study

The primary objective of this program is to analyze the mortality of Hanford workers and particularly to assess the effect of radiation exposure in this population. Communication of both methodology and results is necessary to promote a clear understanding of the problems involved in drawing conclusions about the effects of low-level exposure. Therefore, results of the analyses of our recently updated files as well as a description of our methodology have been presented at a number of forums.

A second objective is to develop methodology appropriate to the analysis of data on low-level chronic exposure experienced by occupational populations. Two areas of research are currently being pursued. The first is a comparative investigation of various statistical approaches to the evaluation of health risks due to occupational exposures; the second is a study of the potentially biasing influence of variables other than radiation.

Updating, and subsequent validation, of the working master file at Pacific Northwest Laboratory was completed in September 1981. Various preliminary analyses have been performed on the resultant file, and procedures used in creating it have been documented.

A paper presenting results of a comparative investigation of various procedures for analyzing occupational exposures has been accepted for publication in the journal Biometrics. Questions of interest concern the advantages and disadvantages of using an external population for comparison, the development of expressions for the power of various procedures for detecting risks of differing magnitudes, and the relative merits of various analytical techniques and approaches to handling dosimetry data.

The impact of variables other than radiation (such as length of employment, job category, employment status, etc.) on mortality has been evaluated using computer software developed specifically for exposure analysis. Such variables are frequently correlated with exposure and can easily bias results when exposure is studied in an occupational setting. Such biases can be particularly severe when deaths are not related to the population at risk (proportional mortality analysis). The results of this research have been reported in a paper submitted to the American Journal of Epidemiology for publication.

Joint efforts with the staff of the Hanford Environmental Health Foundation, who are responsible for the data collection, are under way. This is to evaluate the potential usefulness of the data now in the file or under consideration for future acquisition, the adequacy of quality control procedures, and methods for maintaining files to achieve maximum utility and accessibility. Other questions being explored are the completeness of Social Security Administration death ascertainment and the quality of death certificate diagnosis. The second meeting of the Advisory Committee to the Hanford Health and Mortality Study was held in Richland in October 1981.

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• Radioisotope Customer List

Principal Investigator: J. S. Burlison

The purpose of this program is to prepare and distribute the annual document entitled List of DOE Radioisotope Customers with Summary of Radioisotope Shipments. This document lists the FY 1980 commercial radioisotope production and distribution activities of DOE facilities at Argonne National Laboratory, Pacific Northwest Laboratory, Brookhaven National Laboratory, Hanford Engineering Development Laboratory, Idaho Operations Office, Los Alamos Scientific Laboratory, Mound Facility, Oak Ridge National Laboratory, Savannah River Plant, and UNC Nuclear Industries. The report (PNL-3839) was published in August 1981.

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Fusion

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FUSION

Environmental, health and safety problems associated with the fusion technology cannot be precisely identified because of the early stage of development of this technology. Of probable major concern are problems associated with high-strength magnetic fields, with tritium and tritium-breeding materials, with heat transfer materials, with energetic neutrons and the radioactive activation products resulting from interactions with these neutrons. Some of these problems are common to fission as well as fusion technologies and we have considered, in the Fission section, our studies involving the toxicology of sodium and lithium, which may serve as heat transfer and breeding materials in a fusion reactor. In this section, we consider only studies relating to magnetic-field effects.

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• Biological Effects of Magnetic Fields

Principal Investigator: D. D. Mahlum

Other Investigators: C. S. Abernethy, J. R. Decker, Jr., R. G. Genoway, D. R. Kalkwarf, E. G. Kuffel, D. A. Nelson, J. R. Skalski, J. A. Strand, and C. Veverka, Jr.

Although reports continue to appear in the literature concerning biological effects of magnetic fields, the current data base is extremely weak. This project has as its major thrust the evaluation of a variety of biological systems for their sensitivity to magnetic fields. The systems being explored range from cell culture to pre- and postnatal development in the rat. A major weakness of much of the work reported in the literature is the lack of appropriate controls; this is especially true of whole-animal studies. We have therefore spent a great deal of effort to construct a facility which permits both animals being exposed to the field and sham-exposed animals to be housed under nearly identical conditions. In addition to studies on the effects of magnetic fields on whole-animal systems, another phase of these studies has been the investigation of possible mechanisms by which magnetic fields couple to biological systems.

We report here progress on the new magnetic-field exposure facilities, the effect of magnetic fields on fertilization of trout eggs, and results of molecular studies on the interaction of organic compounds with magnetic fields.

Facilities

A 1200-ft^2 metal building has been renovated so as to house two identical beam-bending magnets (Type 18D36) which were obtained on loan from the Stanford Linear Accelerator Center (SLAC). These magnets, which we previously used at SLAC, have poles 45.7 by 91.4 cm, with a gap of 17.8 cm. This provides a relatively large cavity with a uniform vertical field. In addition, the space on either side of the poles can be used for exposure to gradient fields.

An environmentally controlled unit, described in last year's Annual Report, is placed in each magnet to house the animals. The environmental units are matched in size, lighting, temperature and humidity. The mode of operation is such that one magnet is energized (to provide the exposure field) while the other is not. This permits control animals to be maintained under conditions which are as similar as possible to those encountered by the exposed groups. Either magnet can be energized, thereby allowing the locations of control and exposed groups to be exchanged between magnets, minimizing potential differences between units.

Trout Egg Fertilization Studies

Studies on the effect of magnetic-field exposure on trout development were undertaken because other investigations had shown it to be a sensitive system. Moreover, a large number of individuals can be exposed in a small volume of space. During these studies, evidence was obtained that suggested an enhancing effect of magnetic-field exposure on the fertilization process. Additional studies were therefore performed to verify whether enhancement was real.

Four to six 3-yr-old female fish spawned, yielding 6000 (600 ml) eggs. The eggs were mixed and immediately divided into two equal portions in glass beakers. Sperm (30 ml) from four to six 3-yr-old male fish were gathered in a glass beaker and immediately divided between two vials. Eggs and sperm to be exposed were placed within a 1-T magnetic field for 1 hr. Ice packed around the glass beaker containing eggs or sperm maintained temperature at 12.0 ± 0.5°C, a temperature at which no changes in viability are detected. Control eggs and sperm were sham exposed. Eggs and sperm were then well mixed and allowed to stand for 2 min, after which they were rinsed, reconstituted, and water-hardened for 1 hr. The eggs were then incubated for 7 days and scored for fertility.

Experimental results in Table 1 show that in five independent experiments where eggs alone were exposed to the magnetic field, a significant ($\alpha < 0.01$) enhancement of fertilization occurred. Similarly, Table 2 reveals that three of eight replicates indicated a significant ($\alpha < 0.01$) enhancement when sperm alone were exposed prior to fertilization. It may be expected, then, that statistical analyses from nine independent replicates in Table 3 confirmed enhanced fertilization

TABLE 1. Effect of 1-T Magnetic Field on Fertility of Trout Eggs.

	Fertility, %			
Test No.	Control	Exposed	Z-Statistic	α-Level
B-1	97.25	97.25	0.01	0.99
B-2	93.51	96.03	−4.09	<0.01
B-3	93.23	95.39	−3.39	<0.01
6	87.52	97.14	−11.67	<0.01
9	91.90	91.38	0.57	0.57
Overall			−8.30	<0.01

TABLE 2. Effect of 1-T Magnetic Field on Fertility of Trout Sperm.

	Fertility, %			
Test No.	Control	Exposed	Z-Statistic	α-Level
B-1	97.25	96.45	1.70	0.09
B-2	93.51	93.54	−0.05	0.97
B-3	93.23	93.45	−0.32	0.75
6	87.52	89.72	−2.23	0.03
9	91.90	92.78	−0.10	0.32
D-1	72.47	72.80	−0.21	0.83
D-2	96.33	97.46	−2.12	0.03
D-3	53.58	61.60	−4.57	<0.01
Overall			− 3.11	<0.01

TABLE 3. Effect of 1-T Magnetic Field on Combined Trout Eggs and Sperm.

	Fertility, %			
Test No.	Control	Exposed	Z-Statistic	α-Level
A-1	97.64	98.72	−4.10	<0.01
A-2	97.69	98.55	−3.69	<0.01
A-3	99.07	99.10	−0.15	0.88
A-4	98.00	98.60	−2.36	0.02
B-1	97.25	97.01	0.54	0.59
B-2	93.51	95.95	−3.87	<0.01
B-3	93.23	97.09	−6.58	<0.01
6	87.52	97.08	−11.77	<0.01
9	91.90	93.83	−2.27	0.02
Overall			−11.42	<0.01

($\alpha < 0.01$) when both eggs and sperm were exposed to the magnetic field prior to fertilization. Although not shown, combining analyses of all experiments conducted over a 2-yr period revealed a significant ($\alpha < 0.01$) increase in fertilization success among magnetic field (1-T for 1 hr) exposed groups.

At present, the mechanism of influence of the magnetic field is unknown. We speculate that either the micropyle (aperture in the egg shell for admission of sperm) remains open longer than normal or yolk membrane permeability is altered, either of which, individually or in concert, could account for an enhanced fertilization success. Additional experiments are needed to define the mechanism.

Molecular Biomagnetic Studies

Last year, the diffusion of several organic compounds on thin-layer chromatographic (TLC) plates was reported to be displaced in the direction of the increasing magnetic field; this phenomenon was termed "magnetophoresis" (Annual Report, 1980). One of the compounds, diphenylpicrylhydrazyl (DPPH), was known to be paramagnetic, but the magnetic properties of the others (organic dyes with dissimilar structures) were unknown. Electron-spin-resonance (ESR) measurements have now shown that each of the three dyes examined last year, C.I. Acid Blue 9, C.I Acid Red 14 and C.I. Basic Violet 10, contain paramagnetic centers. Figure 1 shows the ESR spectra of C.I. Acid Blue 9, both in crystalline form and adsorbed on silica gel from a TLC plate. The signal intensities were proportional to the concentration of dye in each sample.

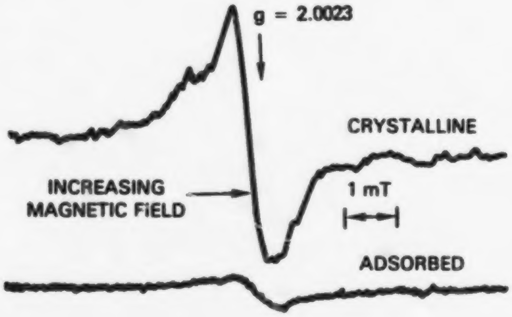

FIGURE 1. ESR Spectra of C.I. Acid Blue 9, in Crystalline Form and Adsorbed on Silica Gel, when Exposed to 1-mT Magnetic Field.

Additional diffusion measurements were made by the method described last year. Briefly, the solute was placed on a TLC plate, and the plate was precisely positioned in a region of measured field and field gradient. Solvent, added to one end of the plate, rose vertically carrying the solute of interest. After measured time periods, the position of the solute on the plate was noted, and any deviation from the vertical projection to the solute's origin was measured. In a magnetic flux density, B, of 0.8 T and a flux-density gradient ($\partial B/\partial \chi$) of 6 T/m, the individual migration rates of DPPH and the organic dyes were 2 μm/s in the direction of increasing flux density. The migration rates also increased linearly with the product $B(\partial B/\partial \chi)$ over the range of 1.5 to 5.0 T^2/m.

Several inorganic paramagnetic ions, e.g., Fe^{3+}, Co^{2+}, Ni^{2+}, were displaced on cellulose TLC plates in the direction of increasing flux density, but diamagnetic species such as Ag^+, Hg^{2+} and dipalmitoylphosphatidylcholine were not (Figure 2). The migration rates of the inorganic paramagnetic ions were only about half those of the organic dyes, but this may be due to the firmer adsorption of inorganic ions on cellulose.

Qualitatively, the results of this study are in agreement with theoretical considerations. The magnetic force on a body is proportional to its magnetic susceptibility, the magnetic flux density and the flux-density gradient (Bates, Modern Magnetism, Fourth Edition, Cambridge Press, 1963). For a paramagnetic substance, the

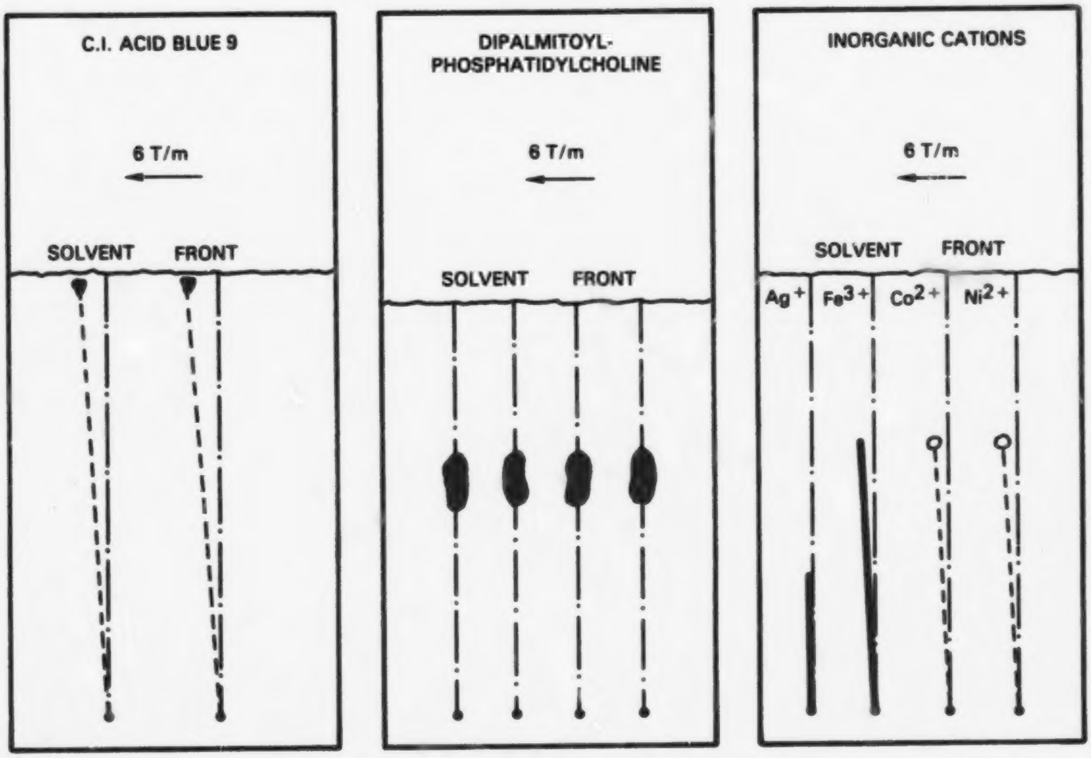

FIGURE 2. Migration Paths of Samples During Their Elution with Solvents on Thin Layers of Cellulose Placed in the Designated Magnetic-Field Gradients. Solvent for C.I. Acid Blue 9 was methanol; solvent for dipalmitoylphosphatidylcholine was $CHCl_3:CH_3OH:CH_3COOH:H_2O$ 50:25:16:4; solvent for inorganic cations was 0.1 M HNO_3 in CH_3OH. In all cases the nominal field strength was B = 0.8 T.

susceptibility is positive, the force is in the direction of increasing flux density and the expected displacement is in the same direction, as found in this study. For a diamagnetic substance, the susceptibility is negative and generally three orders of magnitude less than paramagnetic values. Thus, the lack of detectable displacement for a diamagnetic substance was reasonable.

The results of this study also suggest a possible mechanism for molecular biomagnetic effects. Paramagnetic components in biological systems exposed to magnetic-field gradients will experience a force that tends to move them away from diamagnetic structures. Examples of such components are organic free radicals present as metabolic intermediates and various biopolymers containing transition-metal atoms. Although the migration rates found in this study were attained at high values of $B(\partial B/\partial \chi)$, it is conceivable that even smaller values could cause significant changes in biological structures, such as membranes, that contained loosely bound paramagnetic components.

Multitechnology

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• Metal-Membrane Interactions

Principal Investigator: R. P. Schneider

Other Investigators: H. Drucker and R. A. Lindberg

Technical Assistance: L. M. Butcher

In spite of the ubiquitous, increasing presence of toxic metals derived from the production of energy, little is known of their interactions with membrane uptake and regulatory systems.

The ionic forms of most toxic metals penetrate cell membranes slowly; it therefore seems likely that many of their effects are exerted at the membrane level or are determined by membrane-regulated entry into cells. Information on the interaction of metals with defined membrane functions may therefore be expected to aid in predicting potential effects of trace metals from fossil-fuel utilization and processing.

An important function of membranes is to transmit signals from impermeant molecules in the extracellular space to the cell nucleus, where genes are switched on and off in response to changes in the extracellular medium. Using *Neurospora crassa* as a model of this function, we have shown that cells deprived of phosphorus, nitrogen or carbon secrete an enzyme to digest RNA in response to the presence of extracellular protein. In another study, in which we are investigating the uptake of metals by *N. crassa*, we found that depriving the cells of zinc results in the synthesis of a high-affinity uptake system for zinc that enhances their ability to take up the metal by 1000-fold.

Using different model systems, two studies within this project examine the role of membranes in regulation of metabolism and entry of toxic metals into the cells.

Regulation of Extracellular Ribonuclease Activity in Neurospora Crassa

Control of cell activity and division is, to a large extent, dependent on the transmission of information contained in external impermeant signal molecules through the cell membrane to the nucleus. We have studied the induction of exocellular protease synthesis by external protein inducers in N. crassa as a model system. In this study, we have found that N. crassa can use exogenous ribonucleic acid as a source of nitrogen, carbon, or phosphorus.

A purine catabolic pathway was previously reported to be under control of the nit-2 gene, implying that purines are utilized as a source of nitrogen under conditions of nitrogen insufficiency. To determine the ability of N. crassa to utilize exogenous RNA, we developed a ribonuclease assay that involved the digestion of yeast RNA, followed by determination of non-acid-precipitable products by absorbance at 260 nm. Nitrogen derepression, effected by transfer of mycelia to nitrogen-free media, did not result in extracellular ribonuclease activity (Figure 1). However, the addition of protein (e.g., bovine serum abumin, BSA) to the medium concurrently with limitation of nitrogen resulted in gene derepression. The addition of BSA to repressing media resulted in release of low levels of enzyme activity. This activity was controlled by the nit-2 gene, which is the master control gene of the nitrogen-utilization pathway of the cell.

Figure 1 shows that phosphorus derepression results in the expression of the ribonuclease gene with requirements similar to those of nitrogen derepression. Furthermore, ribonuclease activity was derepressed under carbon limitation (Figure 2). In this case, gene derepression, exogenous protein, and added proteolytic activity were necessary for gene expression. The added requirements of proteolytic activity imply that the ribonuclease gene may respond to the presence of protein in the medium in a manner similar to induction by nonpermeable substrates, previously reported (Annual Reports, 1971-1980) for proteases from N. crassa. Derepression for sulfur, which is not an element of RNA, caused no increase in activity over constitutive levels (Figure 2).

We purified the enzyme solely responsible for the ribonuclease activity from nitrogen-derepressed cultures. This differs from the only previously reported extracellular ribonuclease (from N. crassa) in molecular weight and substrate specificity. The pattern of regulation

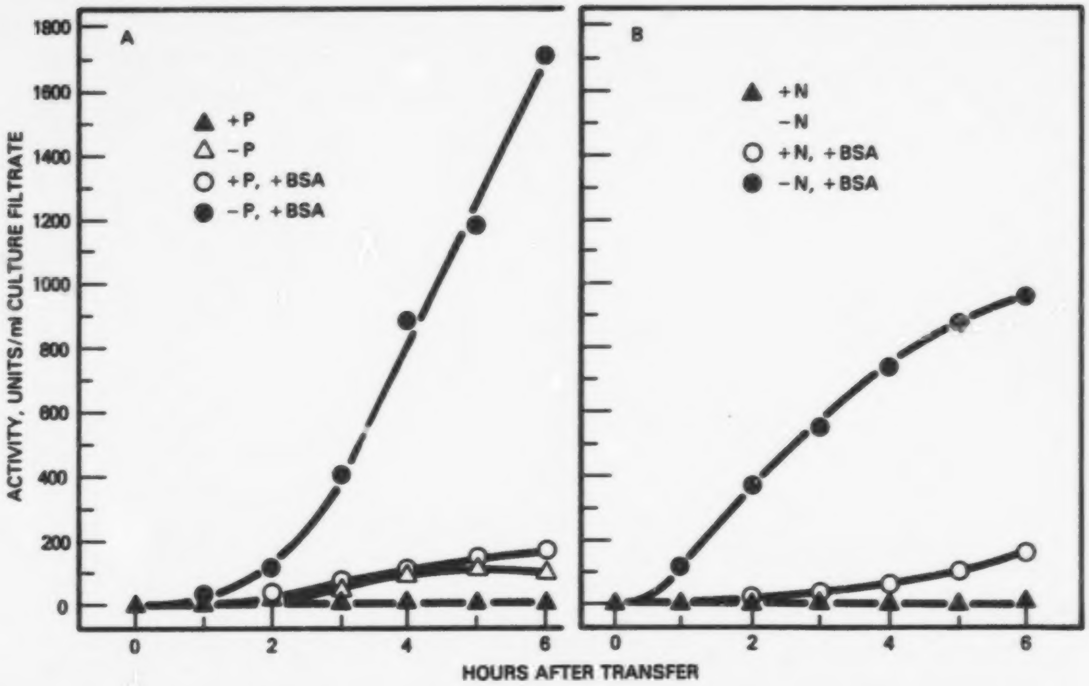

FIGURE 1. Regulation of Extracellular Ribonuclease Activity by Phosphate and Nitrogen. Mycelia were transferred to a selected medium, which was sampled hourly for 6 hr and assayed for ribonuclease activity. Abbreviations: P, phosphate; N, nitrogen; BSA, bovine serum albumin.

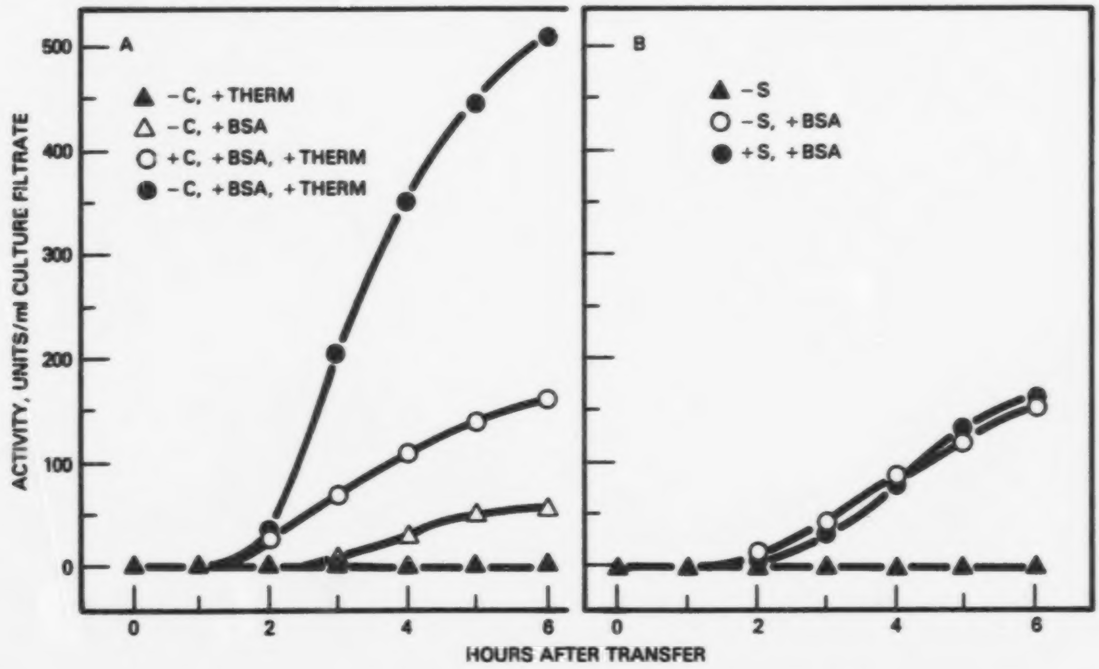

FIGURE 2. Regulation of Extracellular Ribonuclease Activity by Carbon and Sulfur. Procedures were as described for Figure 1. Abbreviations: C, carbon; S, sulfur; Therm, thermolysin.

for this enzyme suggests a cross-pathway regulation involving the nutrilite-utilization pathways for the three nutrilites capable of repression. This is likely accomplished by multiple receptor sites for regulatory macromolecules at a single structural gene, a model similar to those proposed for genes activated by multiple steroid receptor proteins in mammalian cells. Adding to the complexity, it appears that the derepression-mediating macromolecules must be accompanied by another messenger from the membrane that carries information about nonpermeable constituents in the external environment.

Metal Uptake

Neurospora cells are being used as model systems to characterize the uptake of toxic metals into mammalian cells and fungi. Toxic metals are probably transported into cells via uptake systems for nutrient metals, e.g., zinc and iron. The interaction of these metals with nutrients in fungi will aid in predicting their metabolism and mobility in the environment within the context of the availability of nutrient metals.

The interaction between nutrient uptake systems and toxic metal uptake suggests that before studying toxic metal uptake by microorganisms, the regulation and function of the nutrient uptake systems must be described. For example, when exponentially growing suspension cultures of Neurospora were exposed to ^{65}Zn, washed, and the cells assayed for ^{65}Zn, no uptake of radioactivity was observed (Figure 3). However, Neurospora cultures that were previously incubated for 4 hr in Zn-free medium took up ^{65}Zn at a high rate. Because the increase in ability to accumulate Zn did not occur in the presence of an inhibitor of protein synthesis (cycloheximide), we assume that the deprivation

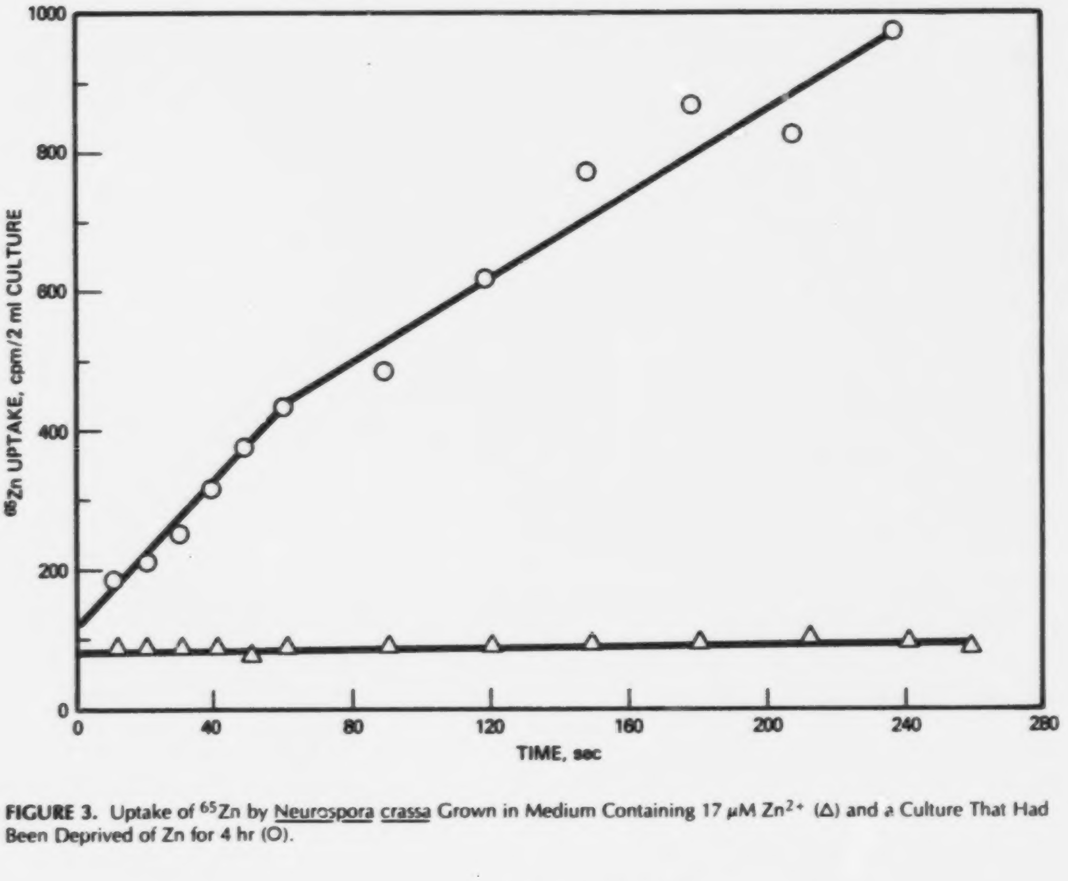

FIGURE 3. Uptake of ^{65}Zn by Neurospora crassa Grown in Medium Containing 17 μM Zn^{2+} (Δ) and a Culture That Had Been Deprived of Zn for 4 hr (O).

of zinc apparently derepressed the synthesis of proteins required for uptake.

The plot of the time course of uptake (Figure 3) shows that it is biphasic, with a rapid component of 60-sec duration, followed by an uptake rate that is linear for more than 15 min. The duration of the more-rapid phase is independent of the uptake rate or the concentration of Zn. We have not ascertained the physiological meaning of the two phases of uptake. The uptake system (except for its biphasic nature), resembles that used to accumulate nutrients required in larger amounts, e.g., phosphorus, glucose, amino acids and potassium. Uptake is a temperature-dependent process. Pretreatment of the cells with a metal chelator inhibits the subsequent uptake of ^{65}Zn, suggesting that a loosely attached protein on the external surface is required for uptake. This also suggests that a protein responsible for Zn binding may be released into the extracellular medium, making the process amenable to biochemical investigation. The uptake of Zn is also blocked by sodium azide, an inhibitor of energy metabolism, demonstrating that it is an active process, i.e., requires energy input. The concentration of Zn required to support one-half maximal uptake (K_m) has not been clearly established but can be estimated to be less than 5 nM, suggesting an extremely high affinity of the receptor for Zn.

The time course of uptake during Zn starvation (Figure 4) shows that deprivation for 1 hr resulted in measurable uptake, but that the maximal rate of increase occurred about 3-4 hr after transfer to Zn-free medium. Due to the presence of Zn stores accumulated during previous growth in high levels of Zn, growth in Zn-free medium continued for 5-7 hr. The cells began to synthesize the uptake system about 4 hr before Zn depletion slowed growth, therefore they responded to decreases in the external or internal concentration of Zn before the shortage was critical.

These studies show that cells growing in the customarily used rich medium are inappropriate for the study of uptake of toxic metals that enter via the Zn uptake system. Populations growing in the natural environment are usually limited by the availability of nutrients and are therefore derepressed. Thus, Zn uptake by derepressed microorganisms provides a more realistic assessment of their potential to incorporate the metal. Future studies will measure uptake of heavy metals and transuranic elements.

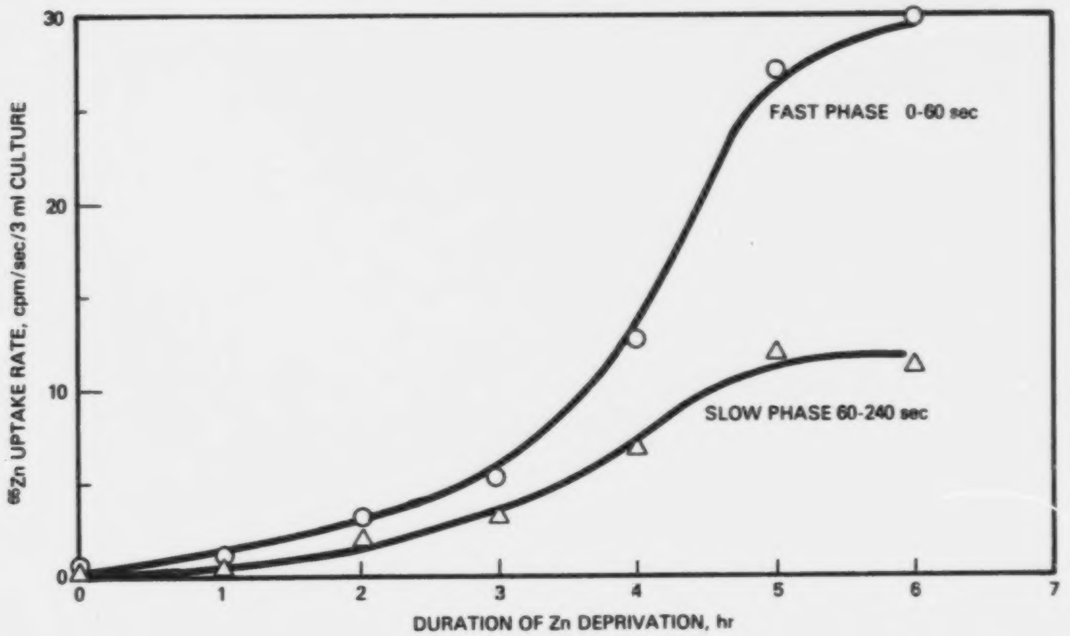

FIGURE 4. The Ability of Neurospora crassa to Accumulate ^{65}Zn as a Function of Time After Transfer to Zn-Free Medium.

• Alveolar Clearance of Inhaled Metal Oxides

Principal Investigator: C. L. Sanders

Other Investigator: K. Rhoads

Metal oxides produced during the combustion of fossil fuels constitute a potential hazard to man. The objectives of this project are to determine the fate of metals deposited in the lung, particularly as influenced by pulmonary cellular dynamics, and their acute and chronic toxicity.

Pulmonary Clearance, Translocation and Excretion of Metal Oxides Following Intratracheal Instillation

Precipitates from aqueous solutions of metal compounds were calcined at 550-750°C to produce high-fired oxides. Young adult rats were given a single intratracheal instillation of 0.5-1.0 ml of the oxide suspension in physiological saline. The doses were 0.35 μg As as As_2O_3 (35 nCi ^{73}As), 13 μg Cd as CdO (15 nCi ^{109}Cd), 1.1 μg Co as Co_3O_4 (100 nCi ^{57}Co), 0.087 μg Se as SeO_4 (1.0 μCi ^{75}Ci), 22 μg V as V_2O_5 (48 nCi ^{48}V) and 0.023 μg Yb as Yb_2O_3 (2.3 nCi ^{169}Yb).

Cadmium, V, Se and As oxides were cleared rapidly from the lung; Yb and Co oxides exhibited moderate retention half-times of 17 and 11 days, respectively. Lung clearance half-times of the other metal oxides ranged from 5 min to 9 hr (Figure 1).

Translocation was mainly to liver and kidney for Cd, liver and bone for As and Se, and bone for V. Cobalt and Yb did not reach high concentrations in any extrapulmonary tissue (Table 1). Excretion at 7 days was greatest for As and V, followed by Se, Co, Yb, and Cd. Ytterbium and Co were excreted almost entirely in feces, whereas As, V, and Se were excreted mainly

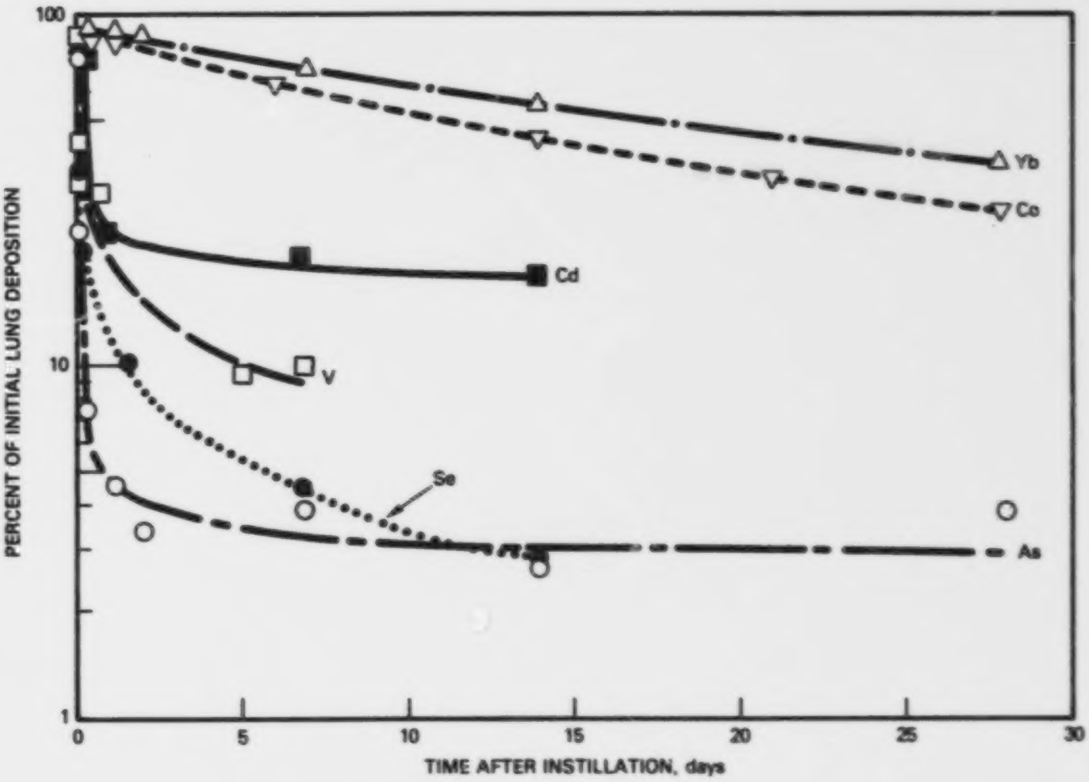

FIGURE 1. Lung Clearance of Intratracheally Instilled Metal Oxides.

TABLE 1. Tissue Burdens and Excretion of Metal Oxides 7 Days After Intratracheal Instillation. (All values are percent of initial lung deposition ± standard deviation; N = 4-6 animals.)

Metal	Tissue			Excreta	
	Liver	Kidney	Skeleton	Urine	Feces
As	9.2 ± 3.7	2.6 ± 0.9	6.9 ± 2.3	27 ± 4.5	14 ± 3.7
Cd	61 ± 2.6	6.3 ± 0.89	1.6 ± 1.7	0.19 ± 0.11	8.1 ± 3.4
Co	0.12 ± 0.079	0.024 ± 0.0035	0.051 ± 0.028	13 ± 1.1	17 ± 0.80
Se	8.6 ± 0.7	2.7 ± 0.1	5.6 ± 1.0	26[a]	8.5[a]
V	4.1 ± 4.5	3.9 ± 0.40	29	33	17
Yb	0.051 ± 0.10	0.13 ± 0.11	0.047 ± 0.93	0.77 ± 0.34	22 ± 5.4

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[a] No 7-day data available; values estimated from clearance curves.

via urine, with a smaller, variable, fecal component. Cobalt excretion was about equal in urine and feces. Whole-body retention (Figure 2) was high for Cd, As and Se, which were translocated rapidly from lung to other organs. This indicated a potential for long-term binding of these metals in extrapulmonary tissues, where they would have their principal effect. In contrast, pathology due to inhalation of Co or Yb would be largely confined to the lung.

It was apparent that the ICRP classifica-

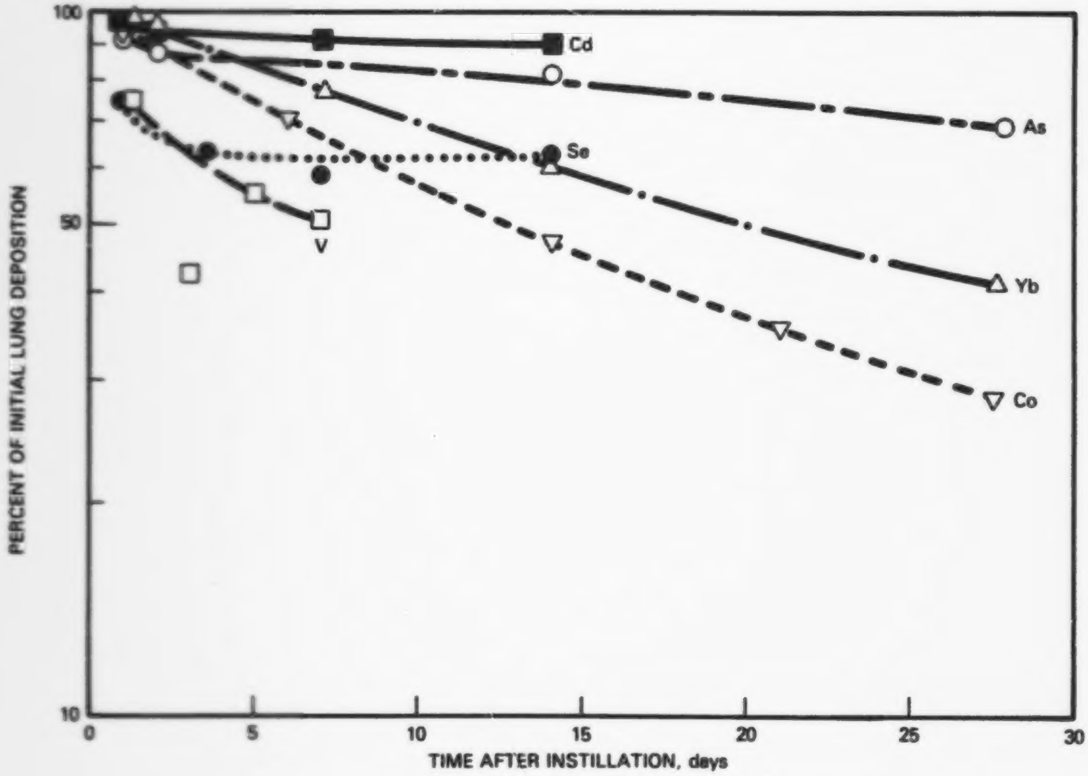

FIGURE 2. Whole-Body Clearance of Intratracheally Instilled Metal Oxides.

tion was not necessarily a good predictor for lung solubility of metal oxides over the time periods covered by this study. For example, cobalt oxide (a class W compound) was cleared more slowly than cadmium oxide (a class Y compound). However, these studies do not cover sufficient time to assess long-term lung retention of small amounts of tissue-bound metal. The implications for toxicity also apply to relatively small amounts of these compounds; larger quantities may cause severe lung damage. The target organs are therefore different for acute and late effects of metals which exhibit lung toxicity at high doses.

Acute Injury to Lung from Cadmium Oxide

Fifty-six young adult rats were given a single intratracheal instillation of approximately one-half the LC_{50} dose (0.16 mg CdO/kg body weight in 0.5 ml physiological saline. The lungs were removed at 1 and 4 hr, or at 1, 2, 7, 14, or 30 days after instillation, when they were compared with those of controls that were instilled with only saline. Fixative-inflated lungs were embedded in glycomethacrylate and examined by transmission and scanning electron microscopy. An acute reaction, seen in the alveoli within 2 days, was characterized by an accumulation of macrophages at 1 day and fibrous material and red blood cells at 2 days (Figures 3 and 4). A marked proliferation of Type II alveolar epithelium was seen at 1 day. In survivors, these reactions to Cd had disappeared, for the most part, by 2 wk.

Fifty-five young rats were given an intratracheal instillation of 0.16 CdO/kg body weight in 0.5 ml physiological saline. The animals were killed at 1 and 4 hr, or at 1, 2, 7 and 14 days after instillation, and the lungs were lavaged (5 x 2 ml) with saline. Total cell count and differential, cell viability assay using trypan-blue dye exclusion, protein content (method of Schwake) and lipid phosphorus content of lavage were determined for CdO and saline-treated control rats. Macrophage viability in intratracheally instilled animals was reduced only at 1 and 4 hr, compared to that of controls. A marked increase in cell count (mostly macrophages) was seen at 1 day, followed by a return to normal by 7-14 days. Lipid phosphorus was moderately increased at 7 days but normal at 14 days. However, protein levels in lavage were markedly increased at 1 day, rapidly falling to normal levels by 14 days (Figure 5). This indicates that the acellular material that accumulated early, in alveoli, is probably the result of inflammation and increased capillary permeability in the alveoli. In contrast, the lipid phosphorus, which appears later, is due to a proliferation

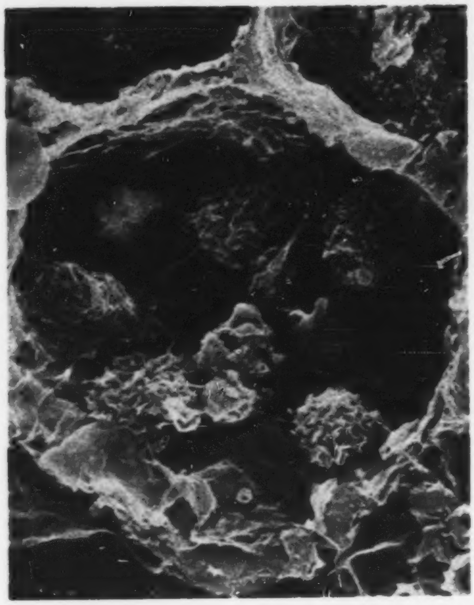

FIGURE 3. Accumulation of Macrophages in Alveoli at 1 Day Following Instillation of CdO.

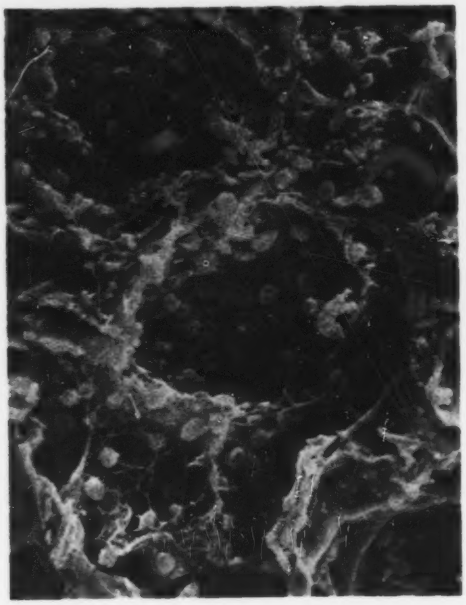

FIGURE 4. Accumulation of Red Blood Cells, Macrophages and Fibrin in Alveoli at 2 Days Following Instillation of CdO.

of Type-II epithelium and extrusion of lamellar bodies.

Carcinogenicity of CdO in Lung

Two hundred male Fischer rats were given either a single intratracheal instillation of saline or 1, 2 or 3 instillations of 25 µg CdO in 0.75 ml saline at monthly intervals. Animals are being held for lifespan observation of tumor frequencies. At 2 yr after instillation of CdO, about 60% of the controls and 60% of CdO-exposed rats have died. Tissues from about half of the dead rats have been histopathologically examined. No apparent significant differences in tumor frequency have been seen among the various treatment groups. Only one primary lung tumor has been seen (adenocarcinoma in a CdO-treated rat).

Volcanic Ash Studies

The effects of Mount St. Helens volcanic ash on the lung and mediastinal lymph nodes of Fischer rats were studied at time intervals of up to 1 yr after intratracheal instillation in 7 consecutive weekly doses for a total deposition of about 80 mg. A Grade 1 fibrosis and moderate lipoproteinosis were seen in the lung at 109 days after initial instillation of ash. Pulmonary reactions to soil particles of comparable particle size were somewhat less than to ash; pulmonary reactions to silica in lung and lymph nodes were severe. Twenty rats were killed at 1 yr after initial instillation of ash. In these rats, the degree of fibrosis and lipoproteinosis appeared to have increased from 109 days to 1 yr after instillation, compared to earlier observations at 109 days (Annual Report, 1980). In some regions of the lung, the lipoproteinosis was very heavy, with large areas exhibiting some accumulation, irrespective of obvious ash presence. The Grade 1 fibrosis (simple pneumoconiosis) seen at 70-109 days after instillation of ash progressed to a Grade 2 fibrosis in limited granulomatous, focal lesions that surrounded aggregations of ash particles. The pulmonary lesions, consisting of macrophage and histocyte accumulations and lipoproteinosis associated with granulomatous reactions, were of sufficient severity to have compromised normal lung function.

A similar comparative study, using Hanford soil particles and ash particles, is in progress.

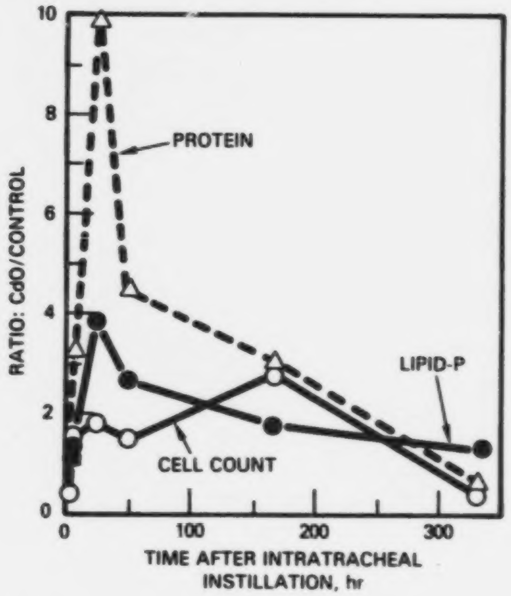

FIGURE 5. Ratio, in CdO-Treated and Control Rats, of Cell Counts, Lipid-P and Protein in Lung Lavages as a Function of Time After Instillation.

Appendix

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APPENDIX

- **Dose-Effect Studies with Inhaled Plutonium in Beagles**

On the following pages data are presented for all dogs employed in current life-span dose effect studies with inhaled $^{239}PuO_2$, $^{238}PuO_2$, and ^{239}Pu nitrate. Information is presented on the estimated initial alveolar deposition, based on external thorax counts and on estimated lung weights (0.011 x body weight) at time of exposure. Information is also provided on the current interpretation of the most prominent clinical-pathological features associated with the death of animals. These data represent information presently available, and are presented as reference material for scientists who desire to follow in detail the progress of these experiments.

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DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
CONTROL	738	F	0	0.00	0.00					149.2*		
CONTROL	740	F	0	0.00	0.00					149.2*		
CONTROL	749	F	0	0.00	0.00					147.9*		
CONTROL	755	M	0	0.00	0.00					147.8*		
CONTROL	766	M	0	0.00	0.00					147.5*		
CONTROL	775	F	0	0.00	0.00					147.1*		
CONTROL	785	M	0	0.00	0.00					146.4*		
CONTROL	789	M	0	0.00	0.00					146.1*		
CONTROL	792	M	0	0.00	0.00				04/28/76		79.5*	Oral Tumor
CONTROL	800	F	0	0.00	0.00					143.3*		
CONTROL	801	M	0	0.00	0.00					143.3*		
CONTROL	811	F	0	0.00	0.00					142.2*		
CONTROL	846	M	0	0.00	0.00					141.4*		
CONTROL	861	M	0	0.00	0.00					141.0*		
CONTROL	868	F	0	0.00	0.00					139.7*		
CONTROL	872	F	0	0.00	0.00					139.6*		
CONTROL	878	M	0	0.00	0.00					137.7*		
CONTROL	882	M	0	0.00	0.00					137.5*		
CONTROL	885	F	0	0.00	0.00					136.9*		
CONTROL	903	F	0	0.00	0.00					134.6*		
CONTROL SACRIFICE	701	F	0	0.00	0.00				04/18/79		121.0*	Sacrificed
CONTROL SACRIFICE	703	M	0	0.00	0.00				03/24/77		96.2*	Sacrificed
CONTROL SACRIFICE	724	M	0	0.00	0.00				03/30/78		107.9*	Sacrificed
D-1 LOWEST	756	M	0	0.00	0.00	13.0	19.5	01/19/71		128.4		
D-1 LOWEST	762	M	0	0.00	0.00	11.5	19.3	01/19/71	01/24/77		72.2	Sacrificed
D-1 LOWEST	847	M	0	0.00	0.00	13.0	18.5	07/06/71		122.8		
D-1 LOWEST	858	M	0	0.00	0.00	13.5	18.2	07/06/71		122.8		
D-1 LOWEST	865	F	0	0.00	0.00	9.0	17.4	07/06/71		122.8		
D-1 LOWEST	879	M	0	0.00	0.00	14.5	17.9	10/07/71		119.8		
D-1 LOWEST	886	F	0	0.00	0.00	10.5	18.2	11/10/71		118.7		
D-1 LOWEST	907	F	0	0.00	0.00	11.5	15.9	11/10/71		118.7		
D-1 LOWEST	825	F	1	0.01	0.12	11.5	18.1	06/08/71		123.8		
D-1 LOWEST	849	F	1	0.01	0.10	10.0	21.3	10/07/71	10/26/72		12.6	Sacrificed
D-1 LOWEST	904	F	1	0.01	0.07	10.0	15.9	11/10/71		118.7		
D-1 LOWEST	832	F	2	0.02	0.22	9.0	16.5	04/26/71		125.2		
D-1 LOWEST	900	M	3	0.02	0.22	13.0	16.0	11/10/71		118.7		
D-1 LOWEST	870	F	4	0.03	0.32	12.0	16.9	07/06/71		122.8		
D-1 LOWEST	899	F	4	0.03	0.31	11.5	16.0	11/10/71	03/29/81		112.6	Hemangiosarcoma, Heart
D-1 LOWEST	867	M	5	0.04	0.41	11.5	17.4	07/06/71		122.8		
D-1 LOWEST	891	M	6	0.04	0.41	14.0	16.0	11/10/71	06/26/81		115.5	Septicemia
D-1 LOWEST	850	F	5	0.05	0.59	8.5	21.3	10/07/71		119.8		
D-1 LOWEST	853	M	8	0.05	0.51	15.0	21.3	10/07/71		119.8		
D-1 LOWEST	875	M	8	0.05	0.54	14.0	16.8	07/06/71	05/21/78		82.5	Kidney:Malignant Lymphoma

* Indicates age in months since birth, all other ages are in months since exposure.

DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
D-1 LOWEST	770	F	6	0.06	0.63	9.5	19.1	01/19/71		128.4		
D-1 LOWEST	788	M	8	0.06	0.62	13.0	18.7	02/09/71		127.7		
D-1 LOWEST	893	M	9	0.06	0.61	14.0	14.9	10/07/71		119.8		
D-1 LOWEST	807	F	8	0.07	0.73	11.0	14.6	02/09/71	07/24/81		125.4	Processing
D-1 LOWEST	841	F	6	0.07	0.75	8.0	17.7	06/08/71		123.8		
D-1 LOWEST	908	M	9	0.07	0.77	11.0	15.9	11/10/71	04/01/80		100.7	Unknown, Pul. Hyalinosis
D-2 LOW	776	M	10	0.07	0.74	13.5	20.2	03/04/71		126.9		
D-2 LOW	842	M	10	0.07	0.77	13.5	18.6	07/06/71		122.8		
D-2 LOW	767	M	10	0.08	0.83	12.0	18.2	12/21/70		129.3		
D-2 LOW	920	M	11	0.08	0.92	12.0	16.0	06/08/72	07/07/72		1.0	Sacrificed
D-2 LOW	862	M	13	0.09	1.00	13.0	17.3	06/08/71		123.8		
D-2 LOW	871	M	13	0.09	0.96	13.5	16.9	07/06/71		122.8		
D-2 LOW	874	M	16	0.11	1.24	13.0	16.8	07/06/71		122.8		
D-2 LOW	754	M	22	0.15	1.69	13.0	19.5	01/19/71	01/10/78		83.7	Status Epilepticus
D-2 LOW	845	F	19	0.15	1.63	11.5	17.6	06/08/71		123.8		
D-2 LOW	748	F	14	0.16	1.75	8.0	19.5	01/19/71	08/19/81		127.0	Processing
D-2 LOW	798	F	16	0.16	1.78	9.0	15.6	02/09/71	08/29/74		42.6	Sacrificed
D-2 LOW	826	F	19	0.17	1.90	10.0	19.1	07/06/71		122.8		
D-2 LOW	831	F	21	0.18	2.00	10.5	17.9	06/08/71		123.8		
D-2 LOW	881	F	19	0.19	2.09	9.0	17.7	10/07/71		119.8		
D-2 LOW	780	F	24	0.22	2.40	10.0	18.2	01/19/71		128.4		
D-2 LOW	859	M	35	0.22	2.41	14.5	18.2	07/06/71		122.8		
D-2 LOW	757	M	36	0.23	2.57	14.0	18.5	12/21/70		129.3		
D-2 LOW	876	F	19	0.23	2.51	7.5	17.9	10/07/71		119.8		
D-2 LOW	806	F	26	0.25	2.74	9.5	15.3	03/04/71		126.9		
D-2 LOW	813	F	32	0.29	3.20	10.0	15.1	03/04/71		126.9		
D-2 LOW	877	F	34	0.29	3.24	10.5	17.9	10/07/71		119.8		
D-2 LOW	769	F	28	0.32	3.50	8.0	18.2	12/21/70	06/23/78		90.1	Ovarian Tumor
D-2 LOW	802	M	40	0.33	3.64	11.0	18.1	04/26/71		125.2		
D-3 MED-LOW	781	F	48	0.38	4.17	11.5	17.3	12/21/70	02/20/81		122.0	Processing
D-3 MED-LOW	771	F	44	0.40	4.40	10.0	19.2	01/20/71		128.3		
D-3 MED-LOW	782	M	62	0.42	4.59	13.5	19.0	02/10/71		127.6		
D-3 MED-LOW	786	M	62	0.42	4.59	13.5	19.5	03/04/71		126.9		
D-3 MED-LOW	752	M	62	0.43	4.77	13.0	18.6	12/21/70	02/22/79		98.1	Lung Tumor, Adrenal Tumor
D-3 MED-LOW	823	M	65	0.44	4.81	13.5	16.8	04/26/71		125.2		
D-3 MED-LOW	883	M	63	0.44	4.85	13.0	17.7	10/07/71		119.8		
D-3 MED-LOW	778	M	74	0.46	5.10	14.5	20.2	03/04/71	08/26/79		101.7	Pul. Thromboembolism
D-3 MED-LOW	838	M	56	0.46	5.09	11.0	17.8	06/08/71		123.8		
D-3 MED-LOW	795	F	54	0.52	5.68	9.5	15.0	01/20/71		128.3		
D-3 MED-LOW	815	M	68	0.52	5.67	12.0	16.8	04/26/71	05/22/73		24.9	Sacrificed
D-3 MED-LOW	851	F	53	0.54	5.89	9.0	21.3	10/07/71		119.8		
D-3 MED-LOW	918	M	74	0.58	6.43	11.5	16.0	06/08/72	07/06/72		0.9	Sacrificed
D-3 MED-LOW	834	F	67	0.68	7.44	9.0	17.8	06/08/71	07/05/79		96.9	Pyometra

* Indicates age in months since birth, all other ages are in months since exposure.

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DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
D-3 MED-LOW	797	F	85	0.70	7.73	11.0	16.4	03/04/71		126.9		
D-3 MED-LOW	848	F	75	0.72	7.94	9.5	21.3	10/07/71		119.8		
D-3 MED-LOW	827	F	89	0.81	8.90	10.0	16.7	04/26/71		125.2		
D-3 MED-LOW	697	M	140	0.85	9.33	15.0	19.5	10/30/70	05/08/80		114.3	Card. Valve insufficiency
D-3 MED-LOW	750	M	118	0.93	10.26	11.5	19.6	01/20/71		128.3		
D-3 MED-LOW	884	M	123	1.12	12.30	10.0	17.8	10/08/71		119.8		
D-3 MED-LOW	844	F	135	1.17	12.86	10.5	17.6	06/08/71		123.8		
D-3 MED-LOW	905	F	127	1.36	14.94	8.5	15.9	11/10/71		118.7		
D-4 MEDIUM	866	M	200	1.35	14.81	13.5	17.4	07/06/71		122.8		
D-4 MEDIUM	809	F	157	1.36	14.95	10.5	15.3	03/04/71	05/28/81		122.8	Processing
D-4 MEDIUM	764	F	158	1.37	15.05	10.5	18.2	12/21/70		129.3		
D-4 MEDIUM	835	F	163	1.48	16.30	10.0	16.4	04/26/71	06/25/78		86.0	Reticulum Cell Sarcoma
D-4 MEDIUM	839	F	189	1.49	16.43	11.5	16.3	04/26/71		125.2		
D-4 MEDIUM	814	F	140	1.50	16.47	8.5	15.1	03/04/71	10/17/79		103.5	Lng Tmr, Thyroid Adenoma
D-4 MEDIUM	836	M	256	1.66	18.29	14.0	17.8	06/08/71	03/16/81		117.3	Lung Tumor
D-4 MEDIUM	819	F	163	1.74	19.18	8.5	18.2	06/08/71		123.8		
D-4 MEDIUM	888	M	274	1.78	19.57	14.0	17.1	10/08/71	07/02/79		92.8	Lung Tumor
D-4 MEDIUM	824	F	227	1.79	19.74	11.5	18.1	06/08/71	01/26/81		115.6	Bronchopneumonia
D-4 MEDIUM	860	M	254	1.85	20.32	12.5	17.3	06/08/71		123.8		
D-4 MEDIUM	833	F	248	2.37	26.11	9.5	16.5	04/26/71		125.2		
D-4 MEDIUM	810	F	302	2.39	26.26	11.5	15.3	03/04/71	09/09/81		126.2	Processing
D-4 MEDIUM	794	M	444	2.60	28.65	15.5	17.7	03/04/71	02/17/81		119.5	Pituitary Tmr, Cushing's
D-4 MEDIUM	854	M	465	2.64	29.06	16.0	21.3	10/08/71		119.8		
D-4 MEDIUM	478	M	298	2.71	29.80	10.0	64.0	10/09/70	10/16/70		0.2	Sacrificed
D-4 MEDIUM	808	F	270	2.73	30.00	9.0	14.6	02/10/71		127.6		
D-4 MEDIUM	805	F	257	3.12	34.27	7.5	18.5	06/08/71		123.8		
D-4 MEDIUM	812	M	438	3.19	35.04	12.5	17.1	04/26/71	11/12/79		102.6	Lung Tumor
D-4 MEDIUM	857	M	486	3.40	37.38	13.0	17.3	06/08/71	07/01/80		108.8	Lung Tumor
D-4 MEDIUM	892	M	494	3.59	39.52	12.5	16.0	11/10/71		118.7		
D-4 MEDIUM	816	M	398	3.62	39.80	10.0	16.8	04/26/71	05/11/71		0.5	Sacrificed
D-4 MEDIUM	777	M	546	3.97	43.68	12.5	20.2	03/04/71	03/26/80		108.7	Lung Tumor
D-4 MEDIUM	803	M	547	4.32	47.57	11.5	18.1	04/26/71	11/10/77		78.5	Interstitial Pneumonitis
D-5 MED-HIGH	787	M	651	4.73	52.08	12.5	19.5	03/04/71	02/08/79		95.2	Lung Tmr, Intestinal Tmr
D-5 MED-HIGH	840	F	703	4.92	54.08	13.0	17.7	06/08/71	04/29/80		106.7	Lung Tumor
D-5 MED-HIGH	727	M	733	5.33	58.64	12.5	18.8	10/26/70	11/10/76		72.5	Lung Tumor
D-5 MED-HIGH	898	F	711	5.39	59.25	12.0	16.0	11/10/71	02/03/81		110.8	Uri Bladr & Lng & Adr Tmr
D-5 MED-HIGH	856	F	818	5.72	62.92	13.0	18.2	07/07/71	05/02/79		93.8	Lung Tumor
D-5 MED-HIGH	759	M	809	6.13	67.42	12.0	18.3	12/21/70	06/02/75		53.4	Lung Tumor
D-5 MED-HIGH	864	F	801	6.62	72.82	11.0	17.4	07/07/71	11/02/79		99.9	Lung Tumor
D-5 MED-HIGH	909	M	737	6.70	73.70	10.0	15.9	11/10/71	06/04/81		114.8	Lung Tumor
D-5 MED-HIGH	734	M	914	6.92	76.17	12.0	19.2	11/10/70	04/01/71		4.7	Sacrificed
D-5 MED-HIGH	837	M	1283	8.04	88.48	14.5	18.8	07/07/71	07/21/77		72.5	Lung Tumor
D-5 MED-HIGH	863	F	980	8.48	93.33	10.5	17.4	07/07/71	10/21/77		75.5	Lung Tumor

* Indicates age in months since birth, all other ages are in months since exposure.

DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/81	DEATH	
D-5 MED-HIGH	820	F	847	8.56	94.11	9.0	18.2	06/08/71	06/01/79		95.8	Lung Tumor
D-5 MED-HIGH	852	F	1187	9.38	103.22	11.5	21.3	10/08/71	02/22/78		76.5	Lung Tumor
D-5 MED-HIGH	880	F	840	9.55	105.00	8.0	17.8	10/08/71	12/04/78		85.9	Lung Tumor
D-5 MED-HIGH	889	F	1089	9.90	108.90	10.0	16.0	11/10/71	09/20/79		94.3	Lng Tmr, Osteoarthropathy
D-5 MED-HIGH	783	M	1394	10.14	111.52	12.5	18.9	02/09/71	12/03/75		57.8	Lung Tumor
D-5 MED-HIGH	804	M	1344	10.18	112.00	12.0	20.5	07/07/71	08/18/74		37.4	Lung Tumor, Rad. Pneum.
D-5 MED-HIGH	873	M	1767	10.71	117.80	15.0	16.8	07/07/71	09/03/76		61.9	Lung Tumor
D-5 MED-HIGH	760	M	1378	10.89	119.83	11.5	19.3	01/20/71	08/15/73		30.8	Radiation Pneumonitis
D-5 MED-HIGH	796	F	1318	11.41	125.52	10.5	15.6	02/09/71	09/17/75		55.2	Lng Tmr, Osteoarthropathy
D-5 MED-HIGH	761	M	1460	12.07	132.73	11.0	19.3	01/20/71	11/02/76		69.4	Lung Tumor
D-5 MED-HIGH	709	M	1726	12.55	138.08	12.5	19.6	11/10/70	03/31/71		4.6	Sacrificed
D-5 MED-HIGH	772	M	1896	14.99	164.87	11.5	19.8	02/09/71	06/26/75		52.5	Lng Tmr, Osteoarthropathy
D-5 MED-HIGH	702	F	1682	15.29	168.20	10.0	19.8	11/10/70	03/31/71		4.6	Sacrificed
D-5 MED-HIGH	739	F	1511	17.17	188.88	8.0	18.5	11/10/70	04/01/71		4.7	Sacrificed
D-6 HIGH	753	F	2448	23.43	257.68	9.5	18.5	12/21/70	10/02/76		69.4	Lung Tumor
D-6 HIGH	817	M	3164	23.97	263.67	12.0	19.2	07/07/71	03/26/73		20.6	Radiation Pneumonitis
D-6 HIGH	829	M	3515	24.58	270.38	13.0	19.1	07/07/71	09/13/73		26.3	Radiation Pneumonitis
D-6 HIGH	890	F	3101	31.32	344.56	9.0	16.0	11/10/71	06/13/74		31.1	Radiation Pneumonitis
D-6 HIGH	435	F	3840	33.25	365.71	10.5	75.5	11/05/70	11/12/70		0.2	Sacrificed
D-6 HIGH	913	M	4900	35.64	392.00	12.5	17.4	07/19/72	08/18/72		1.0	Sacrificed
D-6 HIGH	906	F	6632	63.46	698.11	9.5	15.9	11/10/71	11/22/72		12.4	Radiation Pneumonitis
D-6 HIGH	896	F	5515	66.85	735.33	7.5	16.0	11/10/71	02/12/73		15.1	Radiation Pneumonitis
D-6 HIGH	747	F	7476	97.09	1068.00	7.0	19.6	01/20/71	01/13/72		11.8	Radiation Pneumonitis
D-6 HIGH	910	M	14267	103.76	1141.36	12.5	15.9	11/10/71	10/12/72		11.1	Radiation Pneumonitis

* Indicates age in months since birth, all other ages are in months since exposure.

BEST DOCUMENT AVAILABLE

DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/81	DEATH	COMMENTS ON DEAD DOGS
CONTROL	939	M	0	0.00	0.00					124.9*		
CONTROL	949	F	0	0.00	0.00					124.7*		
CONTROL	978	M	0	0.00	0.00					124.6*		
CONTROL	990	F	0	0.00	0.00				07/08/79		97.4*	Pyometra
CONTROL	996	F	0	0.00	0.00					124.0*		
CONTROL	1005	M	0	0.00	0.00					124.0*		
CONTROL	1007	F	0	0.00	0.00					124.0*		
CONTROL	1024	M	0	0.00	0.00					123.5*		
CONTROL	1038	M	0	0.00	0.00					121.4*		
CONTROL	1045	M	0	0.00	0.00					121.4*		
CONTROL	1054	F	0	0.00	0.00					121.1*		
CONTROL	1061	F	0	0.00	0.00				07/07/81		118.2*	Processing
CONTROL	1093	M	0	0.00	0.00					117.3*		
CONTROL	1097	F	0	0.00	0.00					116.6*		
CONTROL	1112	M	0	0.00	0.00					116.4*		
CONTROL	1116	F	0	0.00	0.00					116.1*		
CONTROL	1186	F	0	0.00	0.00					109.5*		
CONTROL	1197	M	0	0.00	0.00					109.0*		
CONTROL	1209	M	0	0.00	0.00					108.7*		
CONTROL	1225	F	0	0.00	0.00					107.9*		
CONTROL SACRIFICE	966	M	0	0.00	0.00				04/30/77		71.6*	Sacrificed
CONTROL SACRIFICE	1011	F	0	0.00	0.00				06/01/78		83.9*	Sacrificed
CONTROL SACRIFICE	1013	F	0	0.00	0.00				05/29/79		95.8*	Sacrificed
CONTROL SACRIFICE	1087	M	0	0.00	0.00				12/14/76		60.0*	Sacrificed
CONTROL SACRIFICE	1118	M	0	0.00	0.00				01/13/76		47.5*	Sacrificed
CONTROL SACRIFICE	1223	M	0	0.00	0.00				05/15/75		31.9*	Sacrificed
CONTROL SACRIFICE	1227	M	0	0.00	0.00				12/01/76		49.9*	Sacrificed
CONTROL SACRIFICE	1228	M	0	0.00	0.00				10/31/78		72.9*	Sacrificed
D-1 LOWEST	998	M	0	0.00	0.00	10.5	19.6	01/18/73		104.4		
D-1 LOWEST	1003	M	0	0.00	0.00	14.0	19.6	01/18/73		104.4		
D-1 LOWEST	1023	F	0	0.00	0.00	12.5	19.2	01/18/73		104.4		
D-1 LOWEST	1039	M	0	0.00	0.00	11.0	17.0	01/18/73		104.4		
D-1 LOWEST	1044	F	0	0.00	0.00	11.5	17.0	01/18/73		104.4		
D-1 LOWEST	1055	M	0	0.00	0.00	13.0	16.8	01/18/73		104.4		
D-1 LOWEST	1063	M	0	0.00	0.00	14.5	16.7	01/18/73	11/11/80		93.8	Brain Tumor, Heart Tumor
D-1 LOWEST	1105	F	0	0.00	0.00	10.0	16.4	05/31/73		100.0		
D-1 LOWEST	1194	F	0	0.00	0.00	10.5	19.8	04/18/74		89.4		
D-1 LOWEST	1215	M	0	0.00	0.00	15.5	19.3	04/18/74	04/26/77		36.3	Sacrificed
D-1 LOWEST	1230	M	0	0.00	0.00	12.5	18.4	04/18/74		89.4		
D-1 LOWEST	951	M	2	0.01	0.14	14.0	19.3	12/19/72		105.4		
D-1 LOWEST	1008	M	2	0.01	0.15	13.5	19.6	01/18/73		104.4		
D-1 LOWEST	1193	F	2	0.01	0.16	12.5	19.8	04/18/74		89.4		
D-1 LOWEST	959	M	3	0.02	0.22	13.5	19.2	12/19/72		105.4		

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DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
D-1 LOWEST	1069	F	2	0.02	0.24	8.5	18.1	05/31/73		100.0		
D-1 LOWEST	1095	F	2	0.02	0.19	10.5	16.6	05/31/73		100.0		
D-1 LOWEST	921	F	3	0.03	0.31	10.0	19.5	11/30/72	12/27/72		0.9	Sacrificed
D-1 LOWEST	923	F	3	0.03	0.35	8.5	19.5	11/30/72	01/26/73		1.9	Sacrificed
D-1 LOWEST	989	F	3	0.03	0.32	9.5	18.8	12/19/72	03/05/81		98.5	Processing
D-1 LOWEST	925	M	5	0.04	0.40	12.5	19.5	11/30/72	02/27/73		2.9	Sacrificed
D-1 LOWEST	1204	M	6	0.04	0.43	14.0	17.7	02/26/74		91.1		
D-1 LOWEST	970	F	6	0.05	0.55	11.0	19.2	12/19/72	01/04/77		48.5	Sacrificed
D-1 LOWEST	993	F	6	0.05	0.50	12.0	18.8	12/19/72		105.4		
D-1 LOWEST	1106	F	5	0.05	0.50	10.0	16.4	05/31/73		100.0		
D-2 LOW	1065	F	6	0.05	0.60	10.0	18.3	05/31/73		100.0		
D-2 LOW	1082	M	11	0.06	0.69	16.0	18.0	05/31/73	12/04/79		78.1	Paralysis, Spinal Crd Deg
D-2 LOW	1188	M	11	0.06	0.71	15.5	18.4	02/26/74		91.1		
D-2 LOW	1084	M	13	0.07	0.74	17.5	17.5	05/31/73		100.0		
D-2 LOW	1090	F	10	0.08	0.83	12.0	17.3	05/31/73		100.0		
D-2 LOW	1222	M	15	0.10	1.11	13.5	19.0	04/18/74		89.4		
D-2 LOW	971	F	13	0.11	1.24	10.5	19.2	12/19/72		105.4		
D-2 LOW	999	F	11	0.11	1.16	9.5	18.7	12/19/72		105.4		
D-2 LOW	1070	M	22	0.11	1.26	17.5	18.1	05/31/73		100.0		
D-2 LOW	1229	M	16	0.11	1.19	13.5	16.8	02/26/74		91.1		
D-2 LOW	1214	M	17	0.12	1.36	12.5	19.3	04/18/74	05/12/75		12.8	Sacrificed
D-2 LOW	955	M	17	0.14	1.55	11.0	19.2	12/19/72		105.4		
D-2 LOW	1033	M	17	0.14	1.55	11.0	19.1	02/22/73		103.2		
D-2 LOW	1036	F	16	0.14	1.52	10.5	18.2	02/22/73		103.2		
D-2 LOW	1216	M	23	0.16	1.77	13.0	19.3	04/18/74		89.4		
D-2 LOW	1060	F	22	0.18	2.00	11.0	17.8	02/22/73		103.2		
D-2 LOW	981	M	30	0.21	2.31	13.0	19.0	12/19/72		105.4		
D-2 LOW	1046	M	27	0.22	2.45	11.0	18.1	02/22/73		103.2		
D-2 LOW	1050	F	22	0.22	2.44	9.0	18.1	02/22/73		103.2		
D-2 LOW	1078	F	29	0.22	2.42	12.0	18.0	05/31/73		100.0		
D-2 LOW	1207	F	22	0.24	2.59	8.5	17.6	02/26/74		91.1		
D-2 LOW	1196	F	28	0.25	2.80	10.0	17.9	02/26/74		91.1		
D-2 LOW	1189	M	38	0.26	2.81	13.5	20.0	04/18/74	04/25/79		60.2	Sacrificed
D-2 LOW	930	M	38	0.27	2.92	13.0	19.2	11/30/72	12/28/72		0.9	Sacrificed
D-3 MED-LOW	1066	M	54	0.31	3.38	16.0	18.3	05/31/73		100.0		
D-3 MED-LOW	1089	F	41	0.31	3.42	12.0	17.3	05/31/73		100.0		
D-3 MED-LOW	972	F	40	0.33	3.64	11.0	19.2	12/19/72		105.4		
D-3 MED-LOW	1310	M	54	0.34	3.72	14.5	18.5	03/04/75	04/01/77		24.9	Sacrificed
D-3 MED-LOW	1312	M	58	0.34	3.74	15.5	18.5	03/04/75	03/26/79		48.7	Sacrificed
D-3 MED-LOW	1311	M	54	0.36	4.00	13.5	18.5	03/04/75	04/03/78		37.0	Sacrificed
D-3 MED-LOW	1219	F	46	0.40	4.38	10.5	19.0	04/18/74		89.4		
D-3 MED-LOW	1317	M	72	0.41	4.50	16.0	18.1	03/04/75	04/01/77		24.9	Sacrificed
D-3 MED-LOW	1158	M	73	0.43	4.71	15.5	17.7	11/06/73		94.8		

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DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
D-3 MED-LOW	1165	M	76	0.43	4.75	16.0	17.3	11/06/73		94.8		
D-3 MED-LOW	1309	M	60	0.44	4.80	12.5	18.5	03/04/75		78.9		
D-3 MED-LOW	1318	M	67	0.45	4.96	13.5	18.1	03/04/75	03/08/76		12.2	Sacrificed
D-3 MED-LOW	929	F	41	0.50	5.47	7.5	19.2	11/30/72	01/25/73		1.8	Sacrificed
D-3 MED-LOW	1316	M	84	0.53	5.79	14.5	18.1	03/04/75		78.9		
D-3 MED-LOW	960	M	68	0.54	5.91	11.5	19.2	12/19/72	11/07/80		94.6	Malignant Lymphoma
D-3 MED-LOW	1072	M	98	0.54	5.94	16.5	18.1	05/31/73		100.0		
D-3 MED-LOW	1190	F	71	0.54	5.92	12.0	18.1	02/26/74		91.1		
D-3 MED-LOW	926	M	75	0.55	6.00	12.5	19.5	11/30/72	02/28/73		3.0	Sacrificed
D-3 MED-LOW	1315	M	90	0.55	6.00	15.0	18.1	03/04/75	03/31/77		24.9	Sacrificed
D-3 MED-LOW	982	M	76	0.58	6.33	12.0	19.0	12/19/72		105.4		
D-3 MED-LOW	1040	M	84	0.61	6.72	12.5	18.2	02/22/73	03/04/81		96.3	Processing
D-3 MED-LOW	1059	F	71	0.65	7.10	10.0	17.8	02/22/73		103.2		
D-3 MED-LOW	1319	M	99	0.67	7.33	13.5	18.1	03/04/75	03/09/76		12.2	Sacrificed
D-3 MED-LOW	1108	F	84	0.69	7.64	11.0	16.4	05/31/73		100.0		
D-3 MED-LOW	1000	F	70	0.71	7.78	9.0	18.7	12/19/72		105.4		
D-3 MED-LOW	1056	M	97	0.71	7.76	12.5	17.9	02/22/73		103.2		
D-3 MED-LOW	1004	M	116	0.73	8.00	14.5	19.6	01/18/73		104.4		
D-3 MED-LOW	1026	M	116	0.78	8.59	13.5	19.2	01/18/73		104.4		
D-3 MED-LOW	1043	F	98	0.89	9.80	10.0	18.1	02/22/73	09/21/81		102.9	Sacrificed
D-3 MED-LOW	1031	F	76	0.92	10.13	7.5	19.1	02/22/73		103.2		
D-3 MED-LOW	1212	F	111	1.12	12.33	9.0	17.6	02/26/74		91.1		
D-4 MEDIUM	1176	M	129	0.87	9.56	13.5	15.5	10/06/73		95.8		
D-4 MEDIUM	1221	F	124	1.13	12.40	10.0	19.0	04/18/74		89.4		
D-4 MEDIUM	1195	M	228	1.38	15.20	15.0	18.1	02/26/74		91.1		
D-4 MEDIUM	1032	M	162	1.40	15.43	10.5	16.3	11/30/72	12/08/72		0.3	Sacrificed
D-4 MEDIUM	1053	F	148	1.42	15.58	9.5	17.9	02/22/73		103.2		
D-4 MEDIUM	997	M	203	1.60	17.65	11.5	19.6	01/18/73		104.4		
D-4 MEDIUM	991	F	194	1.76	19.40	10.0	18.8	12/19/72		105.4		
D-4 MEDIUM	1177	M	262	1.76	19.41	13.5	16.6	11/06/73		94.8		
D-4 MEDIUM	932	F	216	1.79	19.64	11.0	19.1	11/30/72	01/25/73		1.8	Sacrificed
D-4 MEDIUM	1103	F	260	1.89	20.80	12.5	16.5	05/31/73		100.0		
D-4 MEDIUM	973	F	271	2.24	24.64	11.0	19.2	12/19/72		105.4		
D-4 MEDIUM	931	F	289	2.39	26.27	11.0	19.1	11/30/72	12/28/72		0.9	Sacrificed
D-4 MEDIUM	1091	F	243	2.60	28.59	8.5	17.3	05/31/73		100.0		
D-4 MEDIUM	1114	M	430	2.70	29.66	14.5	16.4	05/31/73		100.0		
D-4 MEDIUM	1062	M	435	2.93	32.22	13.5	17.8	02/22/73		103.2		
D-4 MEDIUM	934	M	454	3.06	33.63	13.5	19.1	11/30/72	03/01/73		3.0	Sacrificed
D-4 MEDIUM	1081	M	541	3.07	33.81	16.0	18.0	05/31/73	01/18/80		79.6	Hemangiosarcoma, Heart
D-4 MEDIUM	1030	F	340	3.25	35.79	9.5	19.1	02/22/73		103.2		
D-4 MEDIUM	1198	M	539	3.50	38.50	14.0	17.9	02/26/74		91.1		
D-4 MEDIUM	952	F	365	3.69	40.56	9.0	19.2	12/19/72		105.4		
D-4 MEDIUM	1166	M	673	4.08	44.87	15.0	17.3	11/06/73		94.8		

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DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
D-4 MEDIUM	1220	F	518	4.28	47.09	11.0	19.0	04/18/74		89.4		
D-4 MEDIUM	992	F	555	4.39	48.26	11.5	18.8	12/19/72		105.4		
D-4 MEDIUM	983	M	617	4.67	51.42	12.0	19.0	12/19/72		105.4		
D-5 MED-HIGH	1191	F	591	4.48	49.25	12.0	19.8	04/18/74	03/21/77		35.1	Interstitial Pneumonitis
D-5 MED-HIGH	1157	M	700	4.71	51.85	13.5	17.7	11/06/73		94.8		
D-5 MED-HIGH	1035	F	571	5.46	60.11	9.5	18.2	02/22/73		103.2		
D-5 MED-HIGH	1192	F	754	6.23	68.55	11.0	18.1	02/26/74		91.1		
D-5 MED-HIGH	1140	M	1014	6.58	72.43	14.0	18.2	11/06/73		94.8		
D-5 MED-HIGH	1071	M	1269	6.79	74.65	17.0	18.1	05/31/73	01/09/81		91.3	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1173	M	1023	7.75	85.25	12.0	17.3	11/06/73		94.8		
D-5 MED-HIGH	1178	M	1125	8.52	93.75	12.0	16.6	11/06/73		94.8		
D-5 MED-HIGH	1047	M	900	8.61	94.74	9.5	18.1	02/22/73		103.2		
D-5 MED-HIGH	1109	F	1119	8.85	97.30	11.5	16.4	05/31/73	08/06/80		86.2	Bone & Lng Tmr, Addison's
D-5 MED-HIGH	1160	F	1344	10.18	112.00	12.0	17.3	11/06/73	09/22/81		94.5	Sacrificed
D-5 MED-HIGH	1211	M	1764	11.06	121.66	14.5	17.6	02/26/74		91.1		
D-5 MED-HIGH	1096	F	1476	12.20	134.18	11.0	16.6	05/31/73	05/08/78		59.2	Addison's Disease
D-5 MED-HIGH	1218	F	1710	12.95	142.50	12.0	17.3	02/26/74	04/24/81		85.9	Bone Tumor
D-5 MED-HIGH	1092	M	1848	13.44	147.84	12.5	17.3	05/31/73	10/23/78		64.8	Bone Tumor
D-5 MED-HIGH	1027	M	2148	13.95	153.43	14.0	19.2	01/18/73	12/01/78		70.4	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1115	F	1885	14.90	163.91	11.5	16.1	05/31/73	07/11/78		61.3	Bone Tumor
D-5 MED-HIGH	974	F	1718	15.62	171.80	10.0	20.2	01/18/73	05/24/78		64.1	Bone Tumor
D-5 MED-HIGH	1079	M	2620	15.88	174.67	15.0	18.0	05/31/73	02/12/78		56.4	Addison's, G.I. Tumor
D-5 MED-HIGH	1058	F	1907	16.51	181.62	10.5	17.8	02/22/73	11/01/79		80.3	Bone Tumor, Adrenal Tumor
D-6 HIGH	1002	M	2907	18.88	207.64	14.0	19.6	01/18/73	01/21/80		84.1	Bone Tumor, Lung Tumor
D-6 HIGH	1057	M	3116	20.98	230.81	13.5	17.9	02/22/73	03/07/79		72.4	Bone Tumor
D-6 HIGH	1009	M	3630	26.40	290.40	12.5	19.6	01/18/73	04/01/78		62.4	Lng Tmr, Osteoarthropathy
D-6 HIGH	1042	F	2959	28.32	311.47	9.5	18.1	02/22/73	11/10/78		68.6	Bone Tumor, Lung Tumor
D-6 HIGH	994	F	3453	31.39	345.30	10.0	19.6	01/18/73	07/04/76		41.5	Addison's Disease
D-6 HIGH	1006	F	3810	31.49	346.36	11.0	19.6	01/18/73	01/18/79		72.0	Bone Tumor, Lung Tumor
D-6 HIGH	975	F	3968	36.07	396.80	10.0	20.2	01/18/73	07/25/78		66.2	Bone Tumor, Lung Tumor
D-6 HIGH	1037	M	4854	44.13	485.40	10.0	18.2	02/22/73	11/21/78		68.9	Bone Tumor
D-6 HIGH	1143	M	7691	53.78	591.62	13.0	18.2	11/06/73	12/05/77		49.0	Bone Tumor, Lung Tumor
D-6 HIGH	1025	M	8479	57.10	628.07	13.5	19.2	01/18/73	03/17/77		49.9	Lung Tumor
D-6 HIGH	1064	M	9453	63.66	700.22	13.5	16.7	01/18/73	04/14/77		50.8	Bone Tumor, Lung Tumor
D-6 HIGH	1162	F	6959	70.29	773.22	9.0	17.3	11/06/73	12/19/78		61.4	Bone Tumor, Addison's
D-6 HIGH	1175	F	6201	75.16	826.80	7.5	16.6	11/06/73	02/24/78		51.6	Lung Tumor

* Indicates age in months since birth, all other ages are in months since exposure.

BEST DOCUMENT AVAILABLE

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INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/81	DEATH	
CONTROL	1356	M	0	0.00	0.00					88.7*		
CONTROL	1365	M	0	0.00	0.00					88.6*		
CONTROL	1376	F	0	0.00	0.00				05/11/80		70.8*	Pneumonia
CONTROL	1388	M	0	0.00	0.00				09/11/81		86.7*	Processing
CONTROL	1393	M	0	0.00	0.00					87.3*		
CONTROL	1405	M	0	0.00	0.00					86.9*		
CONTROL	1409	M	0	0.00	0.00					86.8*		
CONTROL	1418	M	0	0.00	0.00					86.5*		
CONTROL	1425	M	0	0.00	0.00					86.5*		
CONTROL	1450	F	0	0.00	0.00					86.3*		
CONTROL	1455	F	0	0.00	0.00					85.8*		
CONTROL	1483	F	0	0.00	0.00					84.9*		
CONTROL	1509	M	0	0.00	0.00					84.1*		
CONTROL	1516	F	0	0.00	0.00					83.8*		
CONTROL	1525	M	0	0.00	0.00					83.6*		
CONTROL	1526	M	0	0.00	0.00					83.6*		
CONTROL	1528	F	0	0.00	0.00					83.1*		
CONTROL	1543	M	0	0.00	0.00					82.9*		
CONTROL	1563	F	0	0.00	0.00					72.8*		
CONTROL	1572	F	0	0.00	0.00					72.7*		
CONTROL	1577	M	0	0.00	0.00					72.7*		
CONTROL	1584	F	0	0.00	0.00					72.6*		
CONTROL	1594	F	0	0.00	0.00					72.6*		
CONTROL	1608	M	0	0.00	0.00					72.3*		
CONTROL	1633	F	0	0.00	0.00					65.6*		
CONTROL	1638	F	0	0.00	0.00					65.3*		
VEHICLE	1361	M	0	0.00	0.00	8.5	21.0	02/13/76		67.5		
VEHICLE	1381	F	0	0.00	0.00	9.5	19.8	02/13/76		67.5		
VEHICLE	1392	M	0	0.00	0.00	13.0	22.0	04/22/76		65.3		
VEHICLE	1406	M	0	0.00	0.00	13.5	21.6	04/22/76		65.3		
VEHICLE	1412	F	0	0.00	0.00	9.0	19.0	02/13/76		67.5		
VEHICLE	1421	M	0	0.00	0.00	13.0	23.3	06/23/76		63.2		
VEHICLE	1457	F	0	0.00	0.00	12.0	20.6	04/22/76		65.3		
VEHICLE	1491	F	0	0.00	0.00	8.0	21.6	06/23/76		63.2		
VEHICLE	1504	F	0	0.00	0.00	10.0	20.9	06/23/76		63.2		
VEHICLE	1514	M	0	0.00	0.00	14.0	20.9	06/23/76		63.2		
VEHICLE	1524	M	0	0.00	0.00	12.0	21.5	07/27/76		62.1		
VEHICLE	1531	F	0	0.00	0.00	9.0	20.9	07/27/76		62.1		
VEHICLE	1542	M	0	0.00	0.00	12.0	20.8	07/27/76		62.1		
VEHICLE	1566	M	0	0.00	0.00	14.0	18.3	03/15/77		54.5		
VEHICLE	1578	M	0	0.00	0.00	10.5	18.2	03/15/77		54.5		
VEHICLE	1593	F	0	0.00	0.00	11.0	18.0	03/15/77		54.5		
VEHICLE	1601	F	0	0.00	0.00	8.5	18.0	03/15/77		54.5		

* Indicates age in months since birth, all other ages are in months since exposure.

INHALED PLUTONIUM NITRATE IN DOGS

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
VEHICLE	1620	M	0	0.00	0.00	12.0	21.1	12/01/77		46.0		
VEHICLE	1634	F	0	0.00	0.00	10.5	19.6	12/01/77		46.0		
VEHICLE	1651	F	0	0.00	0.00	11.0	19.2	12/01/77		46.0		
D-1 LOWEST	1416	M	0	0.00	0.00	12.0	22.1	05/20/76		64.4		
D-1 LOWEST	1458	F	0	0.00	0.00	10.5	21.5	05/20/76		64.4		
D-1 LOWEST	1489	F	0	0.00	0.00	8.5	20.5	05/20/76		64.4		
D-1 LOWEST	1501	M	0	0.00	0.00	14.0	20.4	05/20/76		64.4		
D-1 LOWEST	1515	M	0	0.00	0.00	13.5	19.8	05/20/76		64.4		
D-1 LOWEST	1573	M	0	0.00	0.00	11.5	19.4	04/19/77		53.4		
D-1 LOWEST	1581	M	0	0.00	0.00	16.5	19.3	04/19/77		53.4		
D-1 LOWEST	1596	M	0	0.00	0.00	14.0	19.2	04/19/77		53.4		
D-1 LOWEST	1600	F	1	0.01	0.11	11.0	19.2	04/19/77		53.4		
D-1 LOWEST	1603	M	2	0.01	0.12	14.0	19.2	04/19/77		53.4		
D-1 LOWEST	1339	F	2	0.02	0.22	9.0	17.5	10/16/75	11/13/75		0.9	Sacrificed
D-1 LOWEST	1519	M	2	0.02	0.18	12.5	19.5	05/20/76		64.4		
D-1 LOWEST	1570	F	2	0.02	0.17	10.5	19.4	04/19/77		53.4		
D-1 LOWEST	1465	F	4	0.03	0.35	12.0	21.0	05/20/76		64.4		
D-1 LOWEST	1470	F	3	0.03	0.29	10.5	21.0	05/20/76		64.4		
D-1 LOWEST	1507	M	4	0.03	0.32	14.0	19.8	05/20/76		64.4		
D-1 LOWEST	1592	F	4	0.03	0.29	13.5	19.2	04/19/77		53.4		
D-1 LOWEST	1607	M	5	0.03	0.35	13.0	19.0	04/19/77		53.4		
D-1 LOWEST	1335	M	5	0.04	0.42	11.5	18.0	10/16/75	11/13/75		0.9	Sacrificed
D-1 LOWEST	1487	F	6	0.04	0.46	13.0	20.5	05/20/76		64.4		
D-1 LOWEST	1583	F	4	0.04	0.40	9.5	19.2	04/19/77		53.4		
D-1 LOWEST	1351	M	7	0.06	0.61	11.0	17.2	10/16/75	11/13/75		0.9	Sacrificed
D-1 LOWEST	1565	F	8	0.06	0.67	11.5	19.4	04/19/77		53.4		
D-2 LOW	1513	M	0	0.00	0.00	11.5	19.8	05/20/76		64.4		
D-2 LOW	1520	M	1	0.01	0.12	10.5	19.5	05/20/76		64.4		
D-2 LOW	1415	M	2	0.02	0.20	11.5	22.2	05/20/76		64.4		
D-2 LOW	1575	M	3	0.02	0.19	14.0	19.4	04/19/77		53.4		
D-2 LOW	1466	F	5	0.03	0.37	14.0	21.0	05/20/76		64.4		
D-2 LOW	1606	F	5	0.04	0.42	12.5	19.0	04/19/77		53.4		
D-2 LOW	1579	M	8	0.05	0.59	14.0	19.3	04/19/77		53.4		
D-2 LOW	1590	F	6	0.05	0.51	12.0	19.2	04/19/77		53.4		
D-2 LOW	1585	F	8	0.06	0.68	12.0	19.2	04/19/77		53.4		
D-2 LOW	1580	F	9	0.07	0.82	11.0	19.3	04/19/77		53.4		
D-2 LOW	1591	M	11	0.07	0.76	15.0	19.2	04/19/77		53.4		
D-2 LOW	1417	M	11	0.08	0.89	12.0	22.1	05/20/76		64.4		
D-2 LOW	1423	M	10	0.08	0.87	11.0	22.1	05/20/76		64.4		
D-2 LOW	1567	M	10	0.08	0.83	12.0	19.4	04/19/77		53.4		
D-2 LOW	1472	F	10	0.09	1.01	10.0	21.0	05/20/76		64.4		
D-2 LOW	1503	F	9	0.09	0.97	9.0	19.8	05/20/76		64.4		
D-2 LOW	1602	M	15	0.09	1.03	14.5	19.2	04/19/77		53.4		

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BEST DOCUMENT AVAILABLE

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INHALED PLUTONIUM NITRATE IN DOGS

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
D-2 LOW	1484	F	11	0.10	1.08	10.0	20.5	05/20/76		64.4		
D-2 LOW	1599	F	10	0.10	1.14	9.0	19.2	04/19/77		53.4		
D-2 LOW	1490	F	16	0.15	1.65	9.5	20.5	05/20/76		64.4		
D-3 MED-LOW	1336	M	21	0.14	1.52	13.5	18.0	10/16/75	11/13/75		0.9	Sacrificed
D-3 MED-LOW	1341	F	19	0.16	1.78	10.5	17.2	10/16/75	11/13/75		0.9	Sacrificed
D-3 MED-LOW	1605	F	25	0.20	2.19	11.5	17.8	03/15/77		54.5		
D-3 MED-LOW	1386	M	34	0.21	2.36	14.5	22.0	04/20/76		65.3		
D-3 MED-LOW	1389	M	27	0.23	2.54	10.5	21.9	04/20/76	05/04/76		0.5	Sacrificed
D-3 MED-LOW	1413	F	29	0.24	2.68	11.0	18.2	01/20/76		68.3		
D-3 MED-LOW	1445	F	34	0.24	2.60	13.0	21.0	04/20/76	05/05/76		0.5	Sacrificed
D-3 MED-LOW	1568	M	46	0.29	3.17	14.5	18.3	03/15/77		54.5		
D-3 MED-LOW	1390	M	43	0.30	3.29	13.0	21.9	04/20/76	05/04/76		0.5	Sacrificed
D-3 MED-LOW	1391	M	54	0.30	3.26	16.5	21.9	04/20/76		65.3		
D-3 MED-LOW	1587	M	53	0.31	3.40	15.5	18.1	03/15/77		54.5		
D-3 MED-LOW	1595	M	50	0.31	3.45	14.5	18.0	03/15/77		54.5		
D-3 MED-LOW	1359	M	50	0.32	3.57	14.0	20.2	01/20/76	01/23/76		0.1	Sacrificed
D-3 MED-LOW	1540	M	54	0.32	3.51	15.5	20.7	07/22/76		62.3		
D-3 MED-LOW	1344	F	41	0.33	3.60	11.5	17.2	10/16/75	11/14/75		1.0	Sacrificed
D-3 MED-LOW	1589	F	41	0.34	3.75	11.0	18.0	03/15/77		54.5		
D-3 MED-LOW	1588	M	50	0.36	3.98	12.5	18.1	03/15/77	03/22/78		12.2	Sacrificed
D-3 MED-LOW	1529	F	43	0.37	4.08	10.5	20.8	07/22/76	10/19/76		2.9	Sacrificed
D-3 MED-LOW	1574	M	46	0.38	4.21	11.0	18.2	03/15/77		54.5		
D-3 MED-LOW	1375	F	50	0.40	4.35	11.5	19.1	01/20/76	01/23/76		0.1	Sacrificed
D-3 MED-LOW	1564	F	40	0.40	4.44	9.0	18.3	03/15/77	03/20/78		12.2	Sacrificed
D-3 MED-LOW	1444	F	49	0.41	4.50	11.0	21.0	04/20/76		65.3		
D-3 MED-LOW	1439	F	53	0.42	4.61	11.5	21.0	04/20/76		65.3		
D-3 MED-LOW	1523	F	55	0.42	4.60	12.0	21.3	07/22/76		62.3		
D-3 MED-LOW	1539	M	65	0.45	4.99	13.0	20.7	07/22/76	10/20/76		3.0	Sacrificed
D-3 MED-LOW	1380	M	63	0.46	5.06	12.5	19.1	01/20/76		68.3		
D-3 MED-LOW	1407	F	50	0.51	5.56	9.0	18.5	01/20/76	01/23/76		0.1	Sacrificed
D-3 MED-LOW	1569	F	58	0.53	5.82	10.0	18.2	03/15/77		54.5		
D-3 MED-LOW	1576	M	70	0.53	5.86	12.0	18.2	03/15/77		54.5		
D-3 MED-LOW	1582	F	57	0.54	5.96	9.5	18.1	03/15/77		54.5		
D-3 MED-LOW	1571	F	68	0.57	6.22	11.0	18.2	03/15/77	03/21/78		12.2	Sacrificed
D-3 MED-LOW	1427	F	68	0.62	6.81	10.0	21.1	04/20/76		65.3		
D-3 MED-LOW	1522	F	78	0.71	7.78	10.0	21.3	07/22/76	10/18/76		2.9	Sacrificed
D-3 MED-LOW	1363	M	85	0.74	8.09	10.5	20.2	01/20/76		68.3		
D-3 MED-LOW	1604	M	85	0.74	8.10	10.5	18.0	03/15/77		54.5		
D-3 MED-LOW	1530	F	72	0.76	8.41	8.5	20.8	07/22/76		62.3		
D-3 MED-LOW	1456	F	61	0.79	8.68	7.0	20.5	04/20/76		65.3		
D-3 MED-LOW	1598	F	93	1.06	11.65	8.0	18.0	03/15/77		54.5		
D-3 MED-LOW	1422	F	99	1.12	12.35	8.0	18.1	01/20/76		68.3		
D-4 MEDIUM	1637	M	192	1.45	15.99	12.0	18.9	11/07/77		46.8		

* Indicates age in months since birth, all other ages are in months since exposure.

INHALED PLUTONIUM NITRATE IN DOGS

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
D-4 MEDIUM	1404	M	260	1.48	16.25	16.0	21.5	04/20/76		65.3		
D-4 MEDIUM	1521	F	205	1.49	16.37	12.5	21.3	07/22/76		62.3		
D-4 MEDIUM	1656	M	211	1.54	16.90	12.5	18.4	11/07/77		46.8		
D-4 MEDIUM	1379	M	278	1.74	19.16	14.5	19.1	01/20/76		68.3		
D-4 MEDIUM	1362	M	267	1.87	20.54	13.0	20.2	01/20/76		68.3		
D-4 MEDIUM	1645	F	257	2.03	22.37	11.5	18.5	11/07/77		46.8		
D-4 MEDIUM	1639	F	248	2.05	22.57	11.0	18.5	11/07/77		46.8		
D-4 MEDIUM	1647	M	294	2.05	22.58	13.0	18.5	11/07/77		46.8		
D-4 MEDIUM	1640	M	307	2.06	22.71	13.5	18.5	11/07/77		46.8		
D-4 MEDIUM	1534	M	295	2.14	23.57	12.5	20.8	07/22/76		62.3		
D-4 MEDIUM	1414	F	233	2.35	25.86	9.0	18.2	01/20/76		68.3		
D-4 MEDIUM	1618	F	277	2.40	26.36	10.5	20.3	11/07/77		46.8		
D-4 MEDIUM	1385	M	373	2.42	26.63	14.0	19.0	01/20/76		68.3		
D-4 MEDIUM	1408	F	331	2.62	28.77	11.5	18.5	01/20/76		68.3		
D-4 MEDIUM	1428	F	378	3.12	34.36	11.0	21.1	04/20/76		65.3		
D-4 MEDIUM	1535	F	345	3.13	34.48	10.0	20.7	07/22/76		62.3		
D-4 MEDIUM	1446	F	354	3.22	35.40	10.0	21.0	04/20/76		65.3		
D-4 MEDIUM	1364	M	463	3.24	35.65	13.0	20.2	01/20/76		68.3		
D-4 MEDIUM	1387	F	345	4.48	49.30	7.0	19.0	01/20/76	08/13/80		54.8	Bone Tumor
D-5 MED-HIGH	1329	F	363	3.30	36.27	10.0	18.0	10/16/75	11/14/75		1.0	Sacrificed
D-5 MED-HIGH	1346	M	656	4.42	48.59	13.5	17.2	10/16/75	11/14/75		1.0	Sacrificed
D-5 MED-HIGH	1648	M	811	5.90	64.90	12.5	18.5	11/07/77		46.8		
D-5 MED-HIGH	1347	F	688	6.95	76.47	9.0	17.2	10/16/75	11/14/75		1.0	Sacrificed
D-5 MED-HIGH	1659	F	990	7.20	79.22	12.5	18.3	11/07/77		46.8		
D-5 MED-HIGH	1636	M	1212	8.48	93.25	13.0	18.9	11/07/77		46.8		
D-5 MED-HIGH	1621	M	1334	8.66	95.26	14.0	20.3	11/07/77		46.8		
D-5 MED-HIGH	1646	F	1061	8.77	96.45	11.0	18.5	11/07/77		46.8		
D-5 MED-HIGH	1429	M	1376	9.62	105.85	13.0	23.2	06/23/76	05/29/81		59.2	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1641	M	1275	9.66	106.24	12.0	18.5	11/07/77		46.8		
D-5 MED-HIGH	1660	M	1518	10.22	112.41	13.5	18.3	11/07/77		46.8		
D-5 MED-HIGH	1508	M	1716	10.76	118.37	14.5	20.9	06/23/76	01/24/80		43.0	Bone Tumor
D-5 MED-HIGH	1655	M	1094	11.05	121.56	9.0	18.4	11/07/77		46.8		
D-5 MED-HIGH	1652	F	1320	12.00	131.95	10.0	18.4	11/07/77		46.8		
D-5 MED-HIGH	1619	F	1490	12.32	135.50	11.0	20.3	11/07/77		46.8		
D-5 MED-HIGH	1512	M	2411	14.61	160.71	15.0	20.9	06/23/76	12/23/79		42.0	Bone Tumor
D-5 MED-HIGH	1419	M	1559	14.92	164.11	9.5	23.3	06/23/76		63.2		
D-5 MED-HIGH	1498	F	2018	16.68	183.45	11.0	21.5	06/23/76		63.2		
D-5 MED-HIGH	1502	F	3008	20.25	222.80	13.5	20.9	06/23/76	01/21/81		55.0	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1485	F	2330	21.18	233.00	10.0	21.7	06/23/76	12/30/80		54.2	Bone Tumor
D-5 MED-HIGH	1471	F	2508	21.71	238.82	10.5	22.1	06/23/76	05/01/79		34.2	Radiation Pneumonitis
D-5 MED-HIGH	1492	F	2473	24.98	274.82	9.0	21.6	06/23/76	10/16/80		51.8	Bone Tumor
D-5 MED-HIGH	1459	F	2645	26.72	293.89	9.0	22.6	06/23/76	09/25/80		51.1	Lung Tumor
D-6 HIGH	1518	M	3565	29.46	324.09	11.0	20.6	06/23/76	12/18/79		41.8	Lung Tumor

* Indicates age in months since birth, all other ages are in months since exposure.

BEST DOCUMENT AVAILABLE

INHALED PLUTONIUM NITRATE IN DOGS

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
D-6 HIGH	1420	M	3840	30.36	333.91	11.5	23.3	06/23/76	07/12/78		24.6	Radiation Pneumonitis
D-6 HIGH	1517	F	5185	49.62	545.79	9.5	20.6	06/23/76	11/02/77		16.3	Radiation Pneumonitis
D-6 HIGH	1510	F	6969	55.09	606.02	11.5	20.9	06/23/76	11/09/77		16.6	Radiation Pneumonitis
D-6 HIGH	1424	M	7681	69.83	768.12	10.0	23.2	06/23/76	08/31/77		14.3	Radiation Pneumonitis

* Indicates age in months since birth, all other ages are in months since exposure.

LOW LEVEL PU-238 NITRATE INHALATION STUDIES

			INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE				MONTHS SINCE INHALATION		
DOSE GROUP	DOG NUMBER	SEX	NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	9/30/81	DEATH	COMMENTS ON DEAD DOGS
CONTROL	1548	M	0	0.00	0.00				12/27/77		31.3*	Sacrificed
CONTROL	1555	M	0	0.00	0.00				12/20/77		30.9*	Sacrificed
D-3 MED-LOW	1552	M	49	0.30	3.27	15.0	18.5	12/07/76	01/05/77		1.0	Sacrificed
D-3 MED-LOW	1553	F	44	0.35	3.83	11.5	18.5	12/07/76	01/04/77		0.9	Sacrificed
D-3 MED-LOW	1557	F	42	0.35	3.82	11.0	18.4	12/07/76	03/22/77		3.4	Sacrificed
D-3 MED-LOW	1550	F	57	0.40	4.38	13.0	18.5	12/07/76	12/14/77		12.2	Sacrificed
D-3 MED-LOW	1545	M	59	0.41	4.54	13.0	18.7	12/07/76	01/05/77		1.0	Sacrificed
D-3 MED-LOW	1546	M	70	0.41	4.52	15.5	18.7	12/07/76	03/22/77		3.4	Sacrificed
D-3 MED-LOW	1549	F	62	0.45	4.96	12.5	18.5	12/07/76	12/10/76		0.1	Sacrificed
D-3 MED-LOW	1554	M	73	0.51	5.62	13.0	18.5	12/07/76	12/10/76		0.1	Sacrificed
D-3 MED-LOW	1551	M	85	0.53	5.86	14.5	18.6	12/07/76	03/22/77		3.4	Sacrificed
D-3 MED-LOW	1547	M	75	0.57	6.25	12.0	18.7	12/07/76	12/15/77		12.3	Sacrificed
D-3 MED-LOW	1558	F	72	0.60	6.55	11.0	18.4	12/07/76	12/16/77		12.3	Sacrificed
D-3 MED-LOW	1544	F	107	0.88	9.73	11.0	18.7	12/07/76	12/10/76		0.1	Sacrificed

* Indicates age in months since birth, all other ages are in months since exposure.

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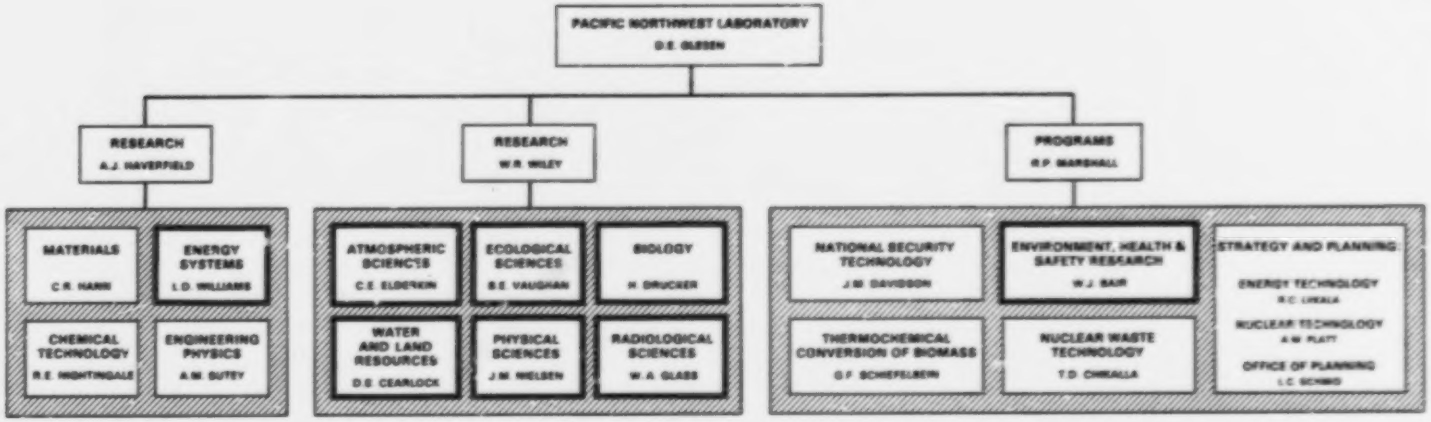
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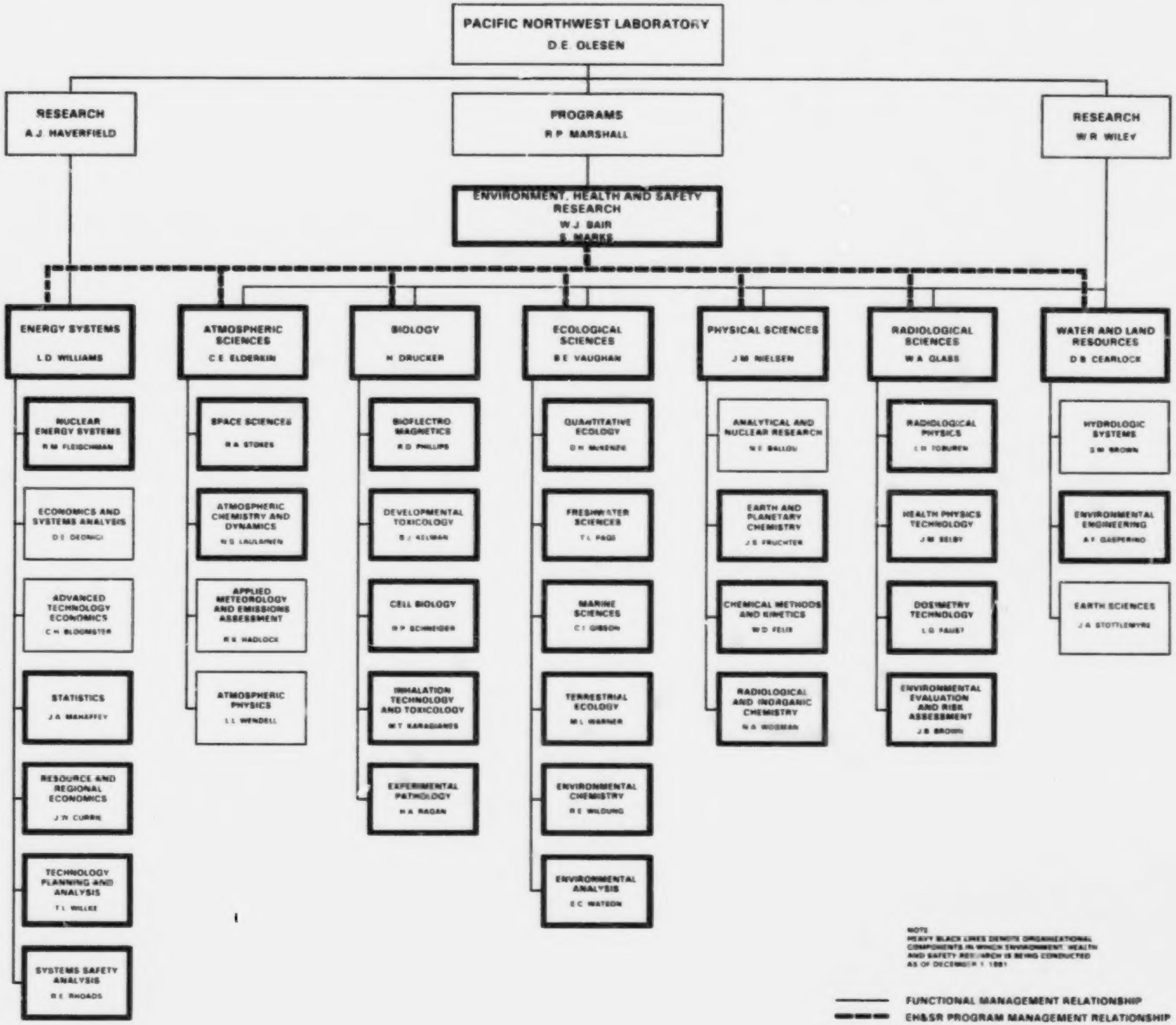
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